Qubit 2.0 Fluorometer Assay Manual

Qubit™ dsDNA BR Assay Kits

For use with the Qubit® 2.0 Fluorometer

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage	Stability
Qubit™ dsDNA BR reagent (Component A)	250 µL or 1.25 mL	200X concentrate in DMSO	• Room temperature • Desiccate • Protect from light	When stored as directed, kits are stable for 6 months
Qubit™ dsDNA BR buffer (Component B)	50 mL or 250 mL	NA	• Room temperature	
Qubit™ dsDNA BR standard #1 (Component C)	1 mL or 5 mL	0 ng/µL in TE buffer	• ≤4°C	
Qubit™ dsDNA BR standard #2 (Component D)	1 mL or 5 mL	100 ng/µL in TE buffer		

NA = Not applicable.

Introduction

The Qubit™ dsDNA BR Assay Kits for use with the Qubit® 2.0 Fluorometer make DNA quantitation easy and accurate. The kit provides concentrated assay reagent, dilution buffer, and pre-diluted DNA standards. Simply dilute the reagent using the buffer provided, add your sample (any volume between 1 µL and 20 µL is acceptable), and read the concentration using the Qubit® 2.0 Fluorometer. The assay is highly selective for double-stranded DNA (dsDNA) over RNA (*Appendix*, Figure 1) and is accurate for initial sample concentration from 100 pg/µL–1,000 ng/µL. The assay is performed at room temperature, and the signal is stable for 3 hours. Common contaminants, such as salts, free nucleotides, solvents, detergents, or protein are well tolerated in the assay (*Appendix,* Table 2). In addition to the Qubit™ dsDNA BR Assay Kits described here, we offer other kits for assaying RNA, protein, and dsDNA at a lower concentration range (*Appendix,* Table 3).

To determine the purity of your sample, use the Qubit™ dsDNA BR Assay Kit together with the Qubit™ RNA Assay Kit. Thease measurements will give you a much better indication of sample purity than that produced by an A_{260}/A_{280} measurement. To measure protein contamination in nucleic acid samples, simply run 1–20 µL of the sample in the Qubit™ protein assay.

Note: All Qubit™ assay kits can also be used with the Qubit® 1.0 Fluorometer.

Before You Begin

Materials Required but Not Provided

- Plastic container (disposable) for mixing the Qubit™ working solution
- Qubit™ assay tubes (500 tubes, Cat. no. Q32856) or Axygen PCR-05-C tubes (VWR, part no. 10011-830)

Storing the Qubit™ dsDNA BR Assay Kits

The Qubit™ dsDNA BR reagent and buffer are designed for room temperature storage. The Qubit™ dsDNA BR reagent is supplied in DMSO, which freezes at temperatures lower than room temperature. Store the DNA standards at 4°C.

Critical Assay Parameters

Assay Temperature

The Qubit™ dsDNA BR assay for the Qubit® 2.0 Fluorometer delivers optimal performance when all solutions are at room temperature (22–28˚C). The Qubit™ assays were designed to be performed at room temperature, as temperature fluctuations can influence the accuracy of the assay (*Appendix*, Figure 2). To minimize temperature fluctuations, store the Qubit™ dsDNA BR reagent and the Qubit™ dsDNA BR buffer at room temperature and insert all assay tubes into the Qubit® 2.0 Fluorometer only for as much time as it takes for the instrument to measure the fluorescence, because the Qubit® 2.0 Fluorometer can raise the temperature of the assay solution significantly, even over a period of a few minutes. Do not hold the assay tubes in your hand before reading, as this will warm the solution and result in a low reading.

Incubation Time

To allow the Qubit™ assay to reach maximum fluorescence, incubate the tubes for the DNA and RNA assays for 2 minutes after mixing the sample or standard with the working solution. After this incubation period, the fluorescence signal is stable for 3 hours at room temperature.

Photobleaching of the Qubit™ Reagent

The Qubit™ reagents exhibit high photostability in the Qubit® 2.0 Fluorometer, showing <0.3% drop in fluorescence after 9 readings and <2.5% drop in fluorescence after 40 readings. It is important to remember, however, that if the assay tube remains in the Qubit® 2.0 Fluorometer for multiple readings, a temporary reduction in fluorescence will be observed as the solution increases in temperature (see *Appendix*, Figure 2). (The temperature inside the Qubit® 2.0 Fluorometer may be as much as 3°C above room temperature after 1 hour.) For this reason, if you want to perform multiple readings of a single tube, you should remove the tube from the instrument and let it equilibrate to room temperature for 30 seconds before taking another reading.

Calibrating the Qubit® 2.0 Fluorometer

For each assay, you have the choice to run a new calibration or to use the values from the previous calibration. As you first use the instrument, you should perform a new calibration each time. As you become familiar with the assays, the instrument, your pipetting accuracy, and significant temperature fluctuations within your laboratory, you should determine the level of comfort you have using the calibration data stored in from the last time the instrument was calibrated. Remember also that the fluorescence signal in the tubes containing standards and the samples is stable for no longer than 3 hours. See Figure 3 in the *Appendix* for an example of the calibration curve used to generate the quantitation results.

Handling and Disposal

We must caution that no data are available addressing the mutagenicity or toxicity of the Qubit™ dsDNA BR reagent (Component A). This reagent is known to bind nucleic acid and is provided as a solution in DMSO. Treat the Qubit™ dsDNA BR reagent with the same safety precautions as all other potential mutagens and dispose of the dye in accordance with local regulations.

Experimental Protocol

Performing the Qubit™ dsDNA BR Assay

The protocol below assumes you will be preparing standards for calibrating the Qubit® 2.0 Fluorometer. If you plan to use the last calibration performed on the instrument, you will need fewer tubes (step 1.1) and less working solution (step 1.3). More detailed instructions on the use of the Qubit® 2.0 Fluorometer (corresponding to steps 1.9–1.15 and 2.1–2.6) can be found in the user manual accompanying the instrument. For sample purity determinations, it is possible to use the Qubit® 2.0 Fluorometer to calculate the amount of dsDNA and RNA in the same sample. Simply perform each assay for your sample.

1.1 Set up the number of 0.5 mL tubes you will need for standards and samples. The Qubit™ dsDNA BR assay requires 2 standards.

Note: Use only thin-wall, clear 0.5 mL PCR tubes. Acceptable tubes include Qubit™ assay tubes (500 tubes, Cat. no. Q32856) or Axygen PCR-05-C tubes (VWR, part no. 10011-830).

1.2 Label the tube lids.

1.3 Make the Qubit™ working solution by diluting the Qubit™ dsDNA BR reagent 1:200 in Qubit™ dsDNA BR buffer. Use a clean plastic tube each time you make Qubit™ working solution. Do not mix the working solution in a glass container.

Note: The final volume in each assay tube must be 200 µL. Each standard tube will require 190 µL of Qubit™ working solution, and each sample tube will require anywhere from 180 µL to 199 µL. Prepare sufficient Qubit™ working solution to accommodate all standards and samples. For example, for 8 samples, prepare enough working solution for the samples and 2 standards: ~200 µL per tube in 10 tubes yields 2 mL of working solution (10 µL of Qubit™ reagent plus 1,990 µL of Qubit™ buffer).

1.4 Load 190 µL of Qubit™ working solution into each of the tubes used for standards.

1.5 Add 10 µL of each Qubit™ standard to the appropriate tube and mix by vortexing 2–3 seconds, being careful not to create bubbles.

Note: Careful pipetting is critical to ensure that exactly 10 µL of each Qubit™ dsDNA BR standard is added to 190 µL of Qubit™ working solution. It is also important to label the lid of each standard tube correctly as calibration of the Qubit® 2.0 Fluorometer requires that the standards be introduced to the instrument in the right order.

1.6 Load Qubit™ working solution into individual assay tubes so that the final volume in each tube after adding sample is 200 µL.

Note: Your sample can be anywhere between 1 µL and 20 µL, therefore, load each assay tube with a volume of Qubit™ working solution anywhere between 180 µL and 199 µL.

1.7 Add each of your samples to assay tubes containing the correct volume of Qubit™ working solution (prepared in step 1.6) and mix by vortexing 2–3 seconds. The final volume in each tube should be 200 µL.

1.8 Allow all tubes to incubate at room temperature for 2 minutes.

1.9 On the Home Screen of the Qubit® 2.0 Fluorometer, press **DNA**, and then select **dsDNA Broad Range** as the assay type. The Standards Screen is automatically displayed.

Note: If you have already performed a calibration for the selected assay, Qubit® 2.0 Fluorometer will prompt you to choose between reading new standards and using the previous calibration. See *Calibrating the Qubit® 2.0 Fluorometer* above for calibration guidelines.

1.10 On the Standards Screen, press **Yes** to run a new calibration or press **No** to use the last calibration.

1.11 If you pressed **No** on the Standards Screen, proceed to step 1.12. If you selected **Yes** to a run new calibration, follow instructions below.

Running a New Calibration

Insert the tube containing Standard #1 in the Qubit® 2.0 Fluorometer, close the lid, and press **Read**. The reading will take approximately 3 seconds.
Remove Standard #1.
Insert the tube containing Standard #2 in the Qubit® 2.0 Fluorometer, close the lid, and press **Read**.
Remove Standard #2.

1.12 If you pressed **No** on the Standards Screen, the Sample Screen will be automatically displayed. Insert a sample tube into the Qubit® 2.0 Fluorometer, close the lid, and press **Read**.

1.13 Upon the completion of the measurement, the result will be displayed on the screen.

Note: The value given by the Qubit® 2.0 Fluorometer at this stage corresponds to the concentration after your sample was diluted into the assay tube. You can record this value and perform the calculation yourself to find out the concentration of your original sample (see *Calculating the Concentration of Your Sample*, below) or the Qubit® 2.0 Fluorometer performs this calculation for you (see *Dilution Calculator*, next page).

1.14 To read the next sample, remove the sample from the Qubit® 2.0 Fluorometer, insert the next sample, and press **Read Next Sample**.

1.15 Repeat sample readings until all samples have been read.

Calculating the Concentration of Your Sample

The Qubit® 2.0 Fluorometer gives values for the Qubit™ dsDNA BR assay in µg/mL. This value corresponds to the concentration after your sample was diluted into the assay tube. To calculate the concentration of your sample, use the following equation:

$$\text{Concentration of your sample} = \text{QF value} \times \left(\frac{200}{x}\right)$$

where:
QF value = the value given by the Qubit® 2.0 Fluorometer
x = the number of microliters of sample you added to the assay tube

This equation generates a result with the same units as the value given by the Qubit® 2.0 Fluorometer (i.e., if the Qubit® 2.0 Fluorometer gave a concentration in µg/mL, the result of the equation will be in µg/mL).

Dilution Calculator

The "Dilution Calculator" feature of the Qubit® 2.0 Fluorometer calculates the concentration of your original sample based on the volume of sample you have added to the assay tube. To have the Qubit® 2.0 Fluorometer perform this calculation for you, follow the instruction below.

2.1 Upon completion of the sample measurement, press **Calculate Stock Conc.** The Dilution Calculator Screen containing the volume roller wheel is displayed.

2.2 Using the volume roller wheel, select the volume of your original sample that you have added to the assay tube. When you stop scrolling, the Qubit® 2.0 Fluorometer calculates the original sample concentration based on the measured assay concentration.

2.3 To change the units in which the original sample concentration is displayed, press **µg/mL**. A pop-up window showing the current unit selection (as indicated by an adjacent red dash) opens.

2.4 Select the unit for your original sample concentration by touching the desired unit in the unit selection pop-up window. To close the unit selection pop-up window, touch anywhere on the screen outside the pop-up.

The Qubit® 2.0 Fluorometer automatically converts the units to your selection once the unit selection pop-up window is closed.

Note: The unit button next to your sample concentration reflects the change in the units (e.g., if you change the unit to pg/µL, the button will display pg/µL).

2.5 To save the data from your calculation to the Qubit® 2.0 Fluorometer, press **Save** on the Dilution Calculator screen. The last calculated value of your measurement will be saved as a .CSV file and tagged with a time and date stamp.

2.6 To exit the Dilution Calculator screen, press any navigator button on the bottom of the screen or **Read Next Sample**.

Note: When you navigate away from the Dilution Calculator screen, the Qubit® 2.0 Fluorometer saves the last values for the sample volume and the units in the Dilution Calculator screen only. Returning to the Dilution Calculator screen displays these last selected values.

Appendix

Selectivity of the Qubit™ dsDNA BR Assay

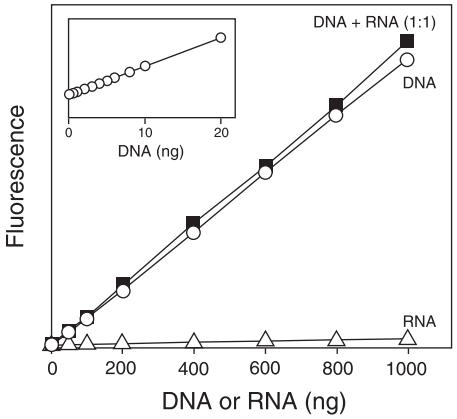

Figure 1. DNA selectivity and sensitivity of the Qubit™ dsDNA BR assay (Q32850, Q32853). Triplicate 10 µL samples of λ DNA (○), *E. coli* rRNA (△), or a 1:1 mixture of DNA and RNA (■) were assayed in the Qubit™ dsDNA BR assay. Fluorescence was measured at 485/530 nm and plotted versus the mass of nucleic acid for the DNA alone or RNA alone, or versus the mass of the DNA component in the 1:1 mixture. The variation (CV) of replicate DNA determinations was ≤3%. The inset, a separate experiment with octuplicate determinations, shows the sensitivity of the assay for DNA. Background fluorescence has not been subtracted.

Effect of Temperature on the Qubit™ dsDNA BR Assay

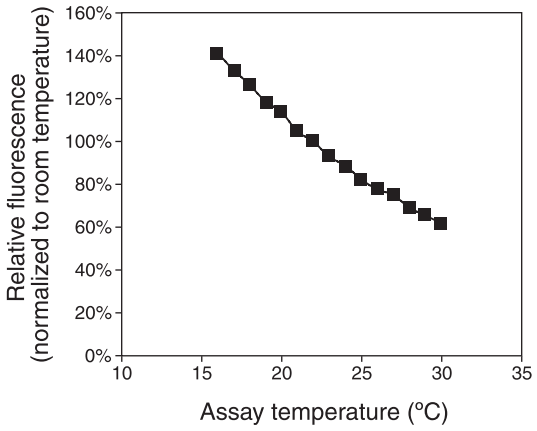

Figure 2. Plot of fluorescence vs. temperature for the Qubit™ dsDNA BR assay. The Qubit™ assays were designed to be performed at room temperature, as temperature fluctuations can influence the accuracy of the assay.

How the Qubit® 2.0 Fluorometer Calculates Concentration

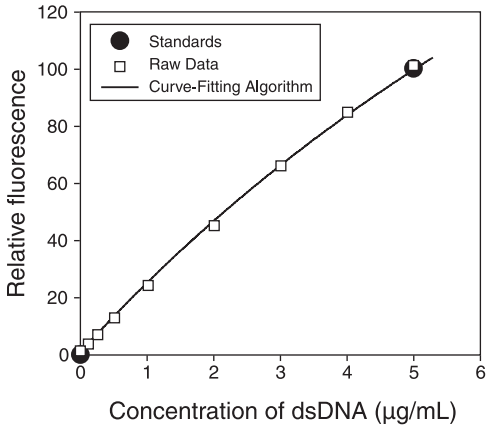

Figure 3. The curve-fitting algorithm used to determine concentration in the Qubit™ dsDNA BR assay. The Qubit® 2.0 Fluorometer generates concentration data based on the relationship between the two standards used in calibration. This plot shows the line corresponding to the curve-fitting algorithm (a modified Hill plot) used in the calculation of concentration data for the Qubit™ dsDNA BR assay. For reference, the positions of the standards and a set of data points from an actual experiment are shown superimposed onto the line, demonstrating that the curve-fitting algorithm gives accurate values for quantitation.

Contaminants Tolerated by the Qubit™ dsDNA BR Assay

Table 2. Effect of contaminants in the Qubit™ dsDNA BR assay, tested over the range 0.01 µg/mL to 5 µg/mL.*

Contaminant	Final Concentration in the Assay	Concentration in 20 µL Sample	Concentration in 10 µL Sample	Result
Sodium chloride	10 mM	100 mM	200 mM	OK †
Magnesium chloride	2 mM	20 mM	40 mM	OK †
Sodium acetate	10 mM	100 mM	200 mM	OK
Ammonium acetate	10 mM	100 mM	200 mM	OK †
Potassium phosphate, pH 7.4	5 mM	50 mM	100 mM	OK †
Ethanol	1%	10%	20%	OK
Phenol	0.1%	1%	2%	OK
Chloroform ‡	0.2%	2%	4%	OK
SDS	0.01%	0.1%	0.2%	OK
Triton X-100	0.001%	0.01%	0.02%	OK †
dNTPs §	100 µM	1 mM	2 mM	OK
BSA	20 µg/mL	200 µg/mL	400 µg/mL	OK †
IgG	10 µg/mL	100 µg/mL	200 µg/mL	OK
RNA	6X	6X	6X	OK
ssDNA	1X	1X	1X	OK
Oligos	3X	3X	3X	OK

* DNA standards were assayed in the presence or absence of contaminants at the indicated final concentrations. Equivalent concentrations (approximate) in 20 µL or 10 µL sample volumes are also listed. In all cases, results are given as OK, usually less than 10% perturbation. † An acceptable result, but with some distortion of the standard curve; for best results, add the same amount of contaminant to the standard samples. ‡ Immiscible. § A mixture of dATP, dCTP, dGTP, and dTTP.

Qubit™ Assay Kits Compatible with the Qubit® 2.0 Fluorometer

A number of fluorescence-based quantitation kits are available for use with the Qubit® 2.0 Fluorometer. Table 3 will help you choose a kit based on the target molecule being measured and the number of assays you require.

Table 3. Qubit™ Assay Kits for use with the Qubit® 2.0 Fluorometer.

Product	Cat. no.	Number of Assays*	Target	Notes
Qubit™ dsDNA BR Assay Kit	Q32850	100	dsDNA	• core range (high confidence): 0.01 µg/mL to 5 µg/mL† • extended range (moderate confidence): 5 µg/mL to 10 µg/mL† • useful for quantitation of genomic and miniprep DNA samples • accurate in the presence of RNA, salts, solvents, proteins, and free nucleotides
Qubit™ dsDNA BR Assay Kit	Q32853	500		
Qubit™ dsDNA HS Assay Kit	Q32851	100	dsDNA	• core range (high confidence): 1 ng/mL to 500 ng/mL† • extended ranges (moderate confidence): 0.5 ng/mL to 1 ng/mL and 500 ng/mL to 600 ng/mL† • useful for quantitation of PCR products, viral DNA, and samples for subcloning • accurate in the presence of RNA, salts, solvents, proteins, and free nucleotides
Qubit™ dsDNA HS Assay Kit	Q32854	500		
Qubit™ ssDNA Assay Kit	Q10212	100	ssDNA	• core range (high confidence): 5 ng/mL to 1,000 ng/mL† • extended ranges (moderate confidence): 1 ng/mL to 5 ng/mL and 1,000 ng/mL to 1,200 ng/mL† • useful for quantitation of oligos, primers, denatured DNA, PCR products • accurate in the presence of salts, urea, solvents, proteins, ATP, and agarose
Qubit™ RNA Assay Kit	Q32852	100	RNA	• core range (high confidence): 25 ng/mL to 500 ng/mL† • extended ranges (moderate confidence): 20 ng/mL to 25 ng/mL and 500 ng/mL to 1,000 ng/mL† • useful for quantitation of samples for microarray, RT-PCR, and Northern blot procedures • accurate in the presence of DNA, salts, solvents, proteins, and free nucleotides
Qubit™ RNA Assay Kit	Q32855	500		
Qubit™ RNA BR Assay Kit	Q10210	100	RNA	• core range (high confidence): 0.1 µg/mL to 5 µg/mL† • extended ranges (moderate confidence): 0.05 µg/mL to 0.1 µg/mL and 5–6 µg/mL† • useful for quantitation of samples for microarray, RT-PCR, and Northern blot procedures • accurate in the presence of DNA, salts, solvents, proteins, and free nucleotides
Qubit™ RNA BR Assay Kit	Q10211	500		
Qubit™ Protein Assay Kit	Q33211	100	protein	• core range (high confidence): 1.25 µg/mL to 25 µg/mL† • extended ranges (moderate confidence): 1 µg/mL to 1.25 µg/mL and 25 µg/mL to 26 µg/mL† • little protein-to-protein difference in signal • accurate in the presence of DTT, β-mercaptoethanol, amino acids, and DNA • signal is stable for 3 hours
Qubit™ Protein Assay Kit	Q33212	500		

*Based on an assay volume of 200 µL. †Concentration ranges refer to the concentration of sample after dilution in the assay tube.

Product List **Current prices may be obtained from our website or from our Customer Service Department.**

Cat. no.	Product Name	Unit Size
Q32850	Qubit™ dsDNA BR Assay Kit, 100 assays *2–1000 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q32853	Qubit™ dsDNA BR Assay Kit 500 assays *2–1000 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Related products		
Q32851	Qubit™ dsDNA HS Assay Kit, 100 assays *0.2–100 ng* *for use with the Qubit® 2.0 Fluorometer	1 kit
Q32854	Qubit™ dsDNA HS Assay Kit, 500 assays *0.2–100 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q10212	Qubit™ ssDNA Assay Kit, 100 assays *1–200 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q10210	Qubit™ RNA BR Assay Kit, 100 assays *20–1000 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q10211	Qubit™ RNA BR Assay Kit, 500 assays *20–1000 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q32852	Qubit™ RNA Assay Kit, 100 assays *5–100 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q32855	Qubit™ RNA Assay Kit, 500 assays *5–100 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q33211	Qubit™ Protein Assay Kit, 100 assays *0.25–5 µg* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q33212	Qubit™ Protein Assay Kit, 500 assays *0.25–5 µg* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q32856	Qubit™ assay tubes *set of 500*	1 set
Q32866	Qubit® 2.0 Fluorometer	each
Q32867	Qubit® 2.0 Fluorometer USB	each
Q32868	Qubit® 2.0 Fluorometer International Power Cord (replacement)	each

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Order Fax: (800) 438-0228

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Further information on Molecular Probes products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Paisley, United Kingdom. All others should contact our Technical Service Department in Eugene, Oregon.

Molecular Probes products are high-quality reagents and materials intended for research purposes only. These products must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Please read the Material Safety Data Sheet provided for each product; other regulatory considerations may apply.

For research use only. Not intended for any animal or human therapeutic or diagnostic use.

Qubit™ dsDNA HS Assay Kits

For use with the Qubit® 2.0 Fluorometer

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage	Stability
Qubit™ dsDNA HS Reagent (Component A)	250 µL or 1.25 mL	200X concentrate in DMSO	• Room temperature • Desiccate • Protect from light	When stored as directed, kits are stable for 6 months.
Qubit™ dsDNA HS Buffer (Component B)	50 mL or 250 mL	NA	• Room temperature	
Qubit™ dsDNA HS Standard #1 (Component C)	1 mL or 5 mL	0 ng/µL in TE buffer	• ≤4°C	
Qubit™ dsDNA HS Standard #2 (Component D)	1 mL or 5 mL	10 ng/µL in TE buffer		

NA = Not applicable.

Introduction

The Qubit™ dsDNA HS Assay Kits for use with the Qubit® 2.0 Fluorometer make DNA quantitation easy and accurate. The kit provides concentrated assay reagent, dilution buffer, and pre-diluted DNA standards. Simply dilute the reagent using the buffer provided, add your sample (any volume between 1 µL and 20 µL is acceptable), and read the concentration using the Qubit® 2.0 Fluorometer. The assay is highly selective for double-stranded DNA (dsDNA) over RNA (*Appendix*, Figure 1) and is accurate for initial sample concentrations from 10 pg/µL to 100 ng/µL. The assay is performed at room temperature, and the signal is stable for 3 hours. Common contaminants, such as salts, free nucleotides, solvents, detergents, or protein are well tolerated in the assay (*Appendix*, Table 2). In addition to the Qubit™ dsDNA HS Assay Kits described here, we offer other kits for assaying RNA, protein, and dsDNA at a higher concentration range (*Appendix*, Table 3).

Note: All Qubit™ assay kits can also be used with the Qubit® 1.0 Fluorometer.

Before You Begin

Materials Required but Not Provided

- Plastic container (disposable) for mixing the Qubit™ working solution (step 1.3)
- Qubit™ assay tubes (500 tubes, Cat. no. Q32856) or Axygen PCR-05-C tubes (VWR, part no. 10011-830)

Storing the Qubit™ dsDNA HS Assay Kits

The Qubit™ dsDNA HS reagent and buffer are designed for room temperature storage. The Qubit™ dsDNA HS reagent is supplied in DMSO, which freezes at temperatures lower than room temperature. Store the DNA standards at 4°C.

Critical Assay Parameters

Assay Temperature

The Qubit™ dsDNA HS assay for the Qubit® 2.0 Fluorometer delivers optimal performance when all solutions are at room temperature (22–28˚C). The Qubit™ assays were designed to be performed at room temperature, as temperature fluctuations can influence the accuracy of the assay (*Appendix*, Figure 2). To minimize temperature fluctuations, store the Qubit™ dsDNA HS reagent and the Qubit™ dsDNA HS buffer at room temperature and insert all assay tubes into the Qubit® 2.0 Fluorometer only for as much time as it takes for the instrument to measure the fluorescence, as the Qubit® 2.0 Fluorometer can raise the temperature of the assay solution significantly, even over a period of a few minutes. Do not hold the assay tubes in your hand before reading, as this will warm the solution and result in a low reading.

Incubation Time

In order to allow the Qubit™ assay to reach optimal fluorescence, incubate the tubes for the DNA and RNA assays for 2 minutes after mixing the sample or standard with the working solution. After this incubation period, the fluorescence signal is stable for 3 hours at room temperature.

Photobleaching of the Qubit™ Reagent

The Qubit™ reagents exhibit high photostability in the Qubit® 2.0 Fluorometer, showing <0.3% drop in fluorescence after 9 readings and <2.5% drop in fluorescence after 40 readings. It is important to remember, however, that if the assay tube remains in the Qubit® 2.0 Fluorometer for multiple readings, a temporary reduction in fluorescence will be observed as the solution increases in temperature (see *Appendix*, Figure 2). (The temperature inside the Qubit® 2.0 Fluorometer may be as much as 3°C above room temperature after 1 hour.) For this reason, if you want to perform multiple readings of a single tube, you should remove the tube from the instrument and let it equilibrate to room temperature for 30 seconds before taking another reading.

Calibrating the Qubit® 2.0 Fluorometer

For each assay, you have the choice to run a new calibration or to use the values from the previous calibration. As you first use the instrument, you should perform a new calibration each time. As you become familiar with the assays, the instrument, your pipetting accuracy, and significant temperature fluctuations within your laboratory, you should determine the level of comfort you have using the calibration data stored in from the last time the instrument was calibrated. Remember also that the fluorescence signal in the tubes containing standards and the samples is stable for no longer than 3 hours. See Figure 3 in the *Appendix* for an example of the calibration curve used to generate the quantitation results.

Handling and Disposal

We must caution that no data are available addressing the mutagenicity or toxicity of the Qubit™ dsDNA HS reagent (Component A). This reagent is known to bind nucleic acid and is provided as a solution in DMSO. Treat the Qubit™ dsDNA HS reagent with the same safety precautions as all other potential mutagens and dispose of the dye in accordance with local regulations.

Experimental Protocol

The protocol below assumes you will be preparing standards for calibrating the Qubit® 2.0 Fluorometer. If you plan to use the last calibration performed on the instrument, you will need fewer tubes (step 1.1) and less working solution (step 1.3). More detailed instructions on the use of the Qubit® 2.0 Fluorometer (corresponding to steps 1.9–1.15 and 2.1–2.6) can be found in the user manual accompanying the instrument.

1.1 Set up the number of 0.5 mL tubes you will need for standards and samples. The Qubit™ dsDNA HS assay requires 2 standards.

Note: Use only thin-wall, clear 0.5 mL PCR tubes. Acceptable tubes include Qubit™ assay tubes (500 tubes, Cat. no. Q32856) or Axygen PCR-05-C tubes (VWR, part no. 10011-830).

1.2 Label the tube lids.

1.3 Make the Qubit™ working solution by diluting the Qubit™ dsDNA HS reagent 1:200 in Qubit™ dsDNA HS buffer. Use a clean plastic tube each time you make Qubit™ working solution. Do not mix the working solution in a glass container.

Note: The final volume in each tube must be 200 µL. Each standard tube will require 190 µL of Qubit™ working solution, and each sample tube will require anywhere from 180 µL to 199 µL. Prepare sufficient Qubit™ working solution to accommodate all standards and samples. For example, for 8 samples, prepare enough working solution for the samples and 2 standards: ~200 µL per tube in 10 tubes yields 2 mL of working solution (10 µL of Qubit™ reagent plus 1,990 µL of Qubit™ buffer).

1.4 Load 190 µL of Qubit™ working solution into each of the tubes used for standards.

1.5 Add 10 µL of each Qubit™ standard to the appropriate tube and mix by vortexing 2–3 seconds, being careful not to create bubbles.

Note: Careful pipetting is critical to ensure that exactly 10 µL of each Qubit™ dsDNA HS standard is added to 190 µL of Qubit™ working solution. It is also important to label the lid of each standard tube correctly as calibration of the Qubit® 2.0 Fluorometer requires that the standards be introduced to the instrument in the right order.

1.6 Load Qubit™ working solution into individual assay tubes so that the final volume in each tube after adding sample is 200 µL.

Note: Your sample can be anywhere between 1 µL and 20 µL, therefore, load each assay tube with a volume of Qubit™ working solution anywhere between 180 µL and 199 µL.

1.7 Add each of your samples to assay tubes containing the correct volume of Qubit™ working solution (prepared in step 1.6) and mix by vortexing 2–3 seconds. The final volume in each tube should be 200 µL.

1.8 Allow all tubes to incubate at room temperature for 2 minutes.

1.9 On the Home Screen of the Qubit® 2.0 Fluorometer, press **DNA**, and then select **dsDNA High Sensitivity** as the assay type. The Standards Screen is automatically displayed.

Note: If you have already performed a calibration for the selected assay, Qubit® 2.0 Fluorometer will prompt you to choose between reading new standards and using the previous calibration. See *Calibrating the Qubit® 2.0 Fluorometer* above for calibration guidelines.

1.10 On the Standards Screen, press **Yes** to run a new calibration or press **No** to use the last calibration.

1.11 If you pressed **No** on the Standards Screen, proceed to step 1.12. If you selected **Yes** to a run new calibration, follow instructions below.

Running a New Calibration

Insert the tube containing Standard #1 in the Qubit® 2.0 Fluorometer, close the lid, and press **Read**. The reading will take approximately 3 seconds.
Remove Standard #1.
Insert the tube containing Standard #2 in the Qubit® 2.0 Fluorometer, close the lid, and press **Read**.
Remove Standard #2.

1.12 If you pressed **No** on the Standards Screen, the Sample Screen will be automatically displayed. Insert a sample tube into the Qubit® 2.0 Fluorometer, close the lid, and press **Read**.

1.13 Upon the completion of the measurement, the result will be displayed on the screen.

Note: The value given by the Qubit® 2.0 Fluorometer at this stage corresponds to the concentration after your sample was diluted into the assay tube. You can record this value and perform the calculation yourself to find out the concentration of your original sample (see *Calculating the Concentration of Your Sample*, below) or the Qubit® 2.0 Fluorometer performs this calculation for you (see *Dilution Calculator*, next page).

1.14 To read the next sample, remove the sample from the Qubit® 2.0 Fluorometer, insert the next sample, and press **Read Next Sample**.

1.15 Repeat sample readings until all samples have been read.

Calculating the Concentration of Your Sample

The Qubit® 2.0 Fluorometer gives values for the Qubit™ dsDNA HS assay in ng/mL. This value corresponds to the concentration after your sample was diluted into the assay tube. To calculate the concentration of your sample, use the following equation:

$$\text{Concentration of your sample} = \text{QF value} \times \left(\frac{200}{x}\right)$$

where:

QF value = the value given by the Qubit® 2.0 Fluorometer

x = the number of microliters of sample you added to the assay tube

This equation generates a result with the same units as the value given by the Qubit® 2.0 Fluorometer (i.e., if the Qubit® 2.0 Fluorometer gave a cncentration in ng/mL, the result of the equation will be in ng/mL).

Dilution Calculator

The "Dilution Calculator" feature of the Qubit® 2.0 Fluorometer calculates the concentration of your original sample based on the volume of sample you have added to the assay tube. To have the Qubit® 2.0 Fluorometer perform this calculation for you, follow the instruction below.

2.1 Upon completion of the sample measurement, press **Calculate Stock Conc.** The Dilution Calculator Screen containing the volume roller wheel is displayed.

2.2 Using the volume roller wheel, select the volume of your original sample that you have added to the assay tube. When you stop scrolling, the Qubit® 2.0 Fluorometer calculates the original sample concentration based on the measured assay concentration.

2.3 To change the units in which the original sample concentration is displayed, press **ng/mL**. A pop-up window showing the current unit selection (as indicated by an adjacent red dash) opens.

2.4 Select the unit for your original sample concentration by touching the desired unit in the unit selection pop-up window. To close the unit selection pop-up window, touch anywhere on the screen outside the pop-up.

The Qubit® 2.0 Fluorometer automatically converts the units to your selection once the unit selection pop-up window is closed.

Note: The unit button next to your sample concentration reflects the change in the units (e.g., if you change the unit to pg/µL, the button will display pg/µL).

2.5 To save the data from your calculation to the Qubit® 2.0 Fluorometer, press **Save** on the Dilution Calculator screen. The last calculated value of your measurement will be saved as a .CSV file and tagged with a time and date stamp.

2.6 To exit the Dilution Calculator Screen, press any navigator button on the bottom of the screen or **Read Next Sample**.

Note: When you navigate away from the Dilution Calculator screen, the Qubit® 2.0 Fluorometer saves the last values for the sample volume and the units in the Dilution Calculator screen only. Returning to the Dilution Calculator screen displays these last selected values.

Appendix

Selectivity of the Qubit™ dsDNA HS Assay

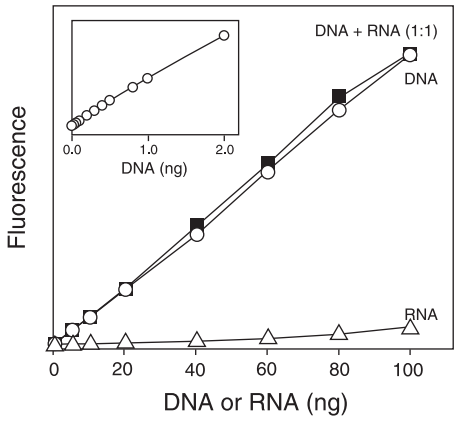

Figure 1. DNA selectivity and sensitivity of the Qubit™ dsDNA HS assay (Q32851, Q32854). Triplicate 10 µL samples of λ DNA (○), *E. coli* rRNA (△), or a 1:1 mixture of DNA and RNA (■) were assayed in the Qubit™ dsDNA HS assay. Fluorescence was measured at 485/530 nm and plotted versus the mass of nucleic acid for the DNA alone or RNA alone, or versus the mass of the DNA component in the 1:1 mixture. The variation (CV) of replicate DNA determinations was ≤2%. The inset, a separate experiment with octuplicate determinations, shows the extreme sensitivity of the assay for DNA. Background fluorescence has not been subtracted.

Effect of Temperature on the Qubit™ dsDNA HS Assay

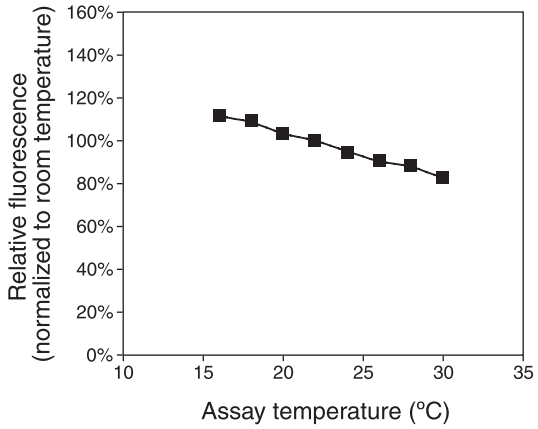

Figure 2. Plot of fluorescence vs. temperature for the Qubit™ dsDNA HS assay. The Qubit™ assays were designed to be performed at room temperature, as temperature fluctuations can influence the accuracy of the assay.

How the Qubit® 2.0 Fluorometer Calculates Concentration

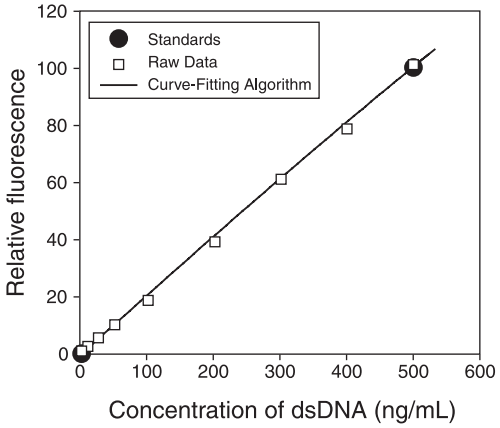

Figure 3. The curve-fitting algorithm used to determine concentration in the Qubit™ dsDNA HS assay. The Qubit® 2.0 Fluorometer generates concentration data based on the relationship between the two standards used in calibration. This plot shows the line corresponding to the curve-fitting algorithm (a modified Hill plot) used in the calculation of concentration data for the Qubit™ dsDNA HS assay. For reference, the positions of the standards and a set of data points from an actual experiment are shown superimposed onto the line, demonstrating that the curve-fitting algorithm gives accurate values for quantitation.

Contaminants Tolerated by the Qubit™ dsDNA HS Assay

Table 2. Effect of contaminants in the Qubit™ dsDNA HS assay, tested over the range of 1 ng/mL to 500 ng/mL.*

Contaminant	Final Concentration in the Assay	Concentration in 20 µL Sample	Concentration in 10 µL Sample	Result
Sodium chloride	50 mM	500 mM	1 M	OK
Magnesium chloride	5 mM	50 mM	100 mM	OK †
Sodium acetate	30 mM	300 mM	600 mM	OK
Ammonium acetate	50 mM	500 mM	1 M	OK
Ethanol	1%	10%	20%	OK
Phenol	0.1%	1%	2%	OK †
Chloroform ‡	1%	10%	20%	OK
SDS	0.01%	0.1%	0.2%	OK
Triton X-100	0.01%	0.1%	0.2%	OK †
dNTPs §	100 µM	1 mM	2 mM	OK
BSA	10 mg/mL	100 mg/mL	200 mg/mL	OK †
IgG	0.5 mg/mL	5 mg/mL	10 mg/mL	OK
RNA	1X	1X	1X	OK
ssDNA	1X	1X	1X	OK
Oligos	1X	1X	1X	OK

* DNA standards were assayed in the presence or absence of contaminants at the indicated final concentrations. Equivalent concentrations (approximate) in 20 µL or 10 µL sample volumes are also listed. In all cases, results are given as OK, usually less than 10% perturbation. † An acceptable result, but with some distortion of the standard curve; for best results, add the same amount of contaminant to the standard samples. ‡ Immiscible. § A mixture of dATP, dCTP, dGTP, and dTTP.

Qubit™ Assay Kits Compatible with the Qubit® 2.0 Fluorometer

A number of fluorescence-based quantitation kits are available for use with the Qubit® 2.0 Fluorometer. Table 3 will help you choose a kit based on the target molecule being measured and the number of assays you require.

Table 3. Qubit™ Assay Kits for use with the Qubit® 2.0 Fluorometer.

Product	Cat. no.	Number of Assays*	Target	Notes
Qubit™ dsDNA BR Assay Kit	Q32850	100	dsDNA	• core range (high confidence): 0.01 µg/mL to 5 µg/mL† • extended range (moderate confidence): 5 µg/mL to 10 µg/mL† • useful for quantitation of genomic and miniprep DNA samples • accurate in the presence of RNA, salts, solvents, proteins, and free nucleotides
Qubit™ dsDNA BR Assay Kit	Q32853	500		
Qubit™ dsDNA HS Assay Kit	Q32851	100	dsDNA	• core range (high confidence): 1 ng/mL to 500 ng/mL† • extended ranges (moderate confidence): 0.5 ng/mL to 1 ng/mL and 500 ng/mL to 600 ng/mL† • useful for quantitation of PCR products, viral DNA, and samples for subcloning • accurate in the presence of RNA, salts, solvents, proteins, and free nucleotides
Qubit™ dsDNA HS Assay Kit	Q32854	500		
Qubit™ ssDNA Assay Kit	Q10212	100	ssDNA	• core range (high confidence): 5 ng/mL to 1,000 ng/mL† • extended ranges (moderate confidence): 1 ng/mL to 5 ng/mL and 1,000 ng/mL to 1,200 ng/mL† • useful for quantitation of oligos, primers, denatured DNA, PCR products • accurate in the presence of salts, urea, solvents, proteins, ATP, and agarose
Qubit™ RNA Assay Kit	Q32852	100	RNA	• core range (high confidence): 25 ng/mL to 500 ng/mL† • extended ranges (moderate confidence): 20 ng/mL to 25 ng/mL and 500 ng/mL to 1,000 ng/mL† • useful for quantitation of samples for microarray, RT-PCR, and Northern blot procedures • accurate in the presence of DNA, salts, solvents, proteins, and free nucleotides
Qubit™ RNA Assay Kit	Q32855	500		
Qubit™ RNA BR Assay Kit	Q10210	100	RNA	• core range (high confidence): 0.1 µg/mL to 5 µg/mL† • extended ranges (moderate confidence): 0.05 µg/mL to 0.1 µg/mL and 5–6 µg/mL† • useful for quantitation of samples for microarray, RT-PCR, and Northern blot procedures • accurate in the presence of DNA, salts, solvents, proteins, and free nucleotides
Qubit™ RNA BR Assay Kit	Q10211	500		
Qubit™ Protein Assay Kit	Q33211	100	protein	• core range (high confidence): 1.25 µg/mL to 25 µg/mL† • extended ranges (moderate confidence): 1 µg/mL to 1.25 µg/mL and 25 µg/mL to 26 µg/mL† • little protein-to-protein difference in signal • accurate in the presence of DTT, β-mercaptoethanol, amino acids, and DNA • signal is stable for 3 hours
Qubit™ Protein Assay Kit	Q33212	500		

*Based on an assay volume of 200 µL. †Concentration ranges refer to the concentration of sample after dilution in the assay tube.

Product List **Current prices may be obtained from our website or from our Customer Service Department.**

Cat. no.	Product Name	Unit Size
Q32851	Qubit™ dsDNA HS Assay Kit, 100 assays *0.2–100 ng* *for use with the Qubit® 2.0 Fluorometer	1 kit
Q32854	Qubit™ dsDNA HS Assay Kit, 500 assays *0.2–100 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Related products		
Q32850	Qubit™ dsDNA BR Assay Kit, 100 assays *2–1000 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q32853	Qubit™ dsDNA BR Assay Kit 500 assays *2–1000 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q10212	Qubit™ ssDNA Assay Kit, 100 assays *1–200 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q10210	Qubit™ RNA BR Assay Kit, 100 assays *20–1000 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q10211	Qubit™ RNA BR Assay Kit, 500 assays *20–1000 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q32852	Qubit™ RNA Assay Kit, 100 assays *5–100 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q32855	Qubit™ RNA Assay Kit, 500 assays *5–100 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q33211	Qubit™ Protein Assay Kit, 100 assays *0.25–5 µg* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q33212	Qubit™ Protein Assay Kit, 500 assays *0.25–5 µg* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q32856	Qubit™ assay tubes *set of 500*	1 set
Q32866	Qubit® 2.0 Fluorometer	each
Q32867	Qubit® 2.0 Fluorometer USB	each
Q32868	Qubit® 2.0 Fluorometer International Power Cord (replacement)	each

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Qubit™ ssDNA Assay Kit

For use with the Qubit® 2.0 Fluorometer

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage	Stability
Qubit™ ssDNA Reagent (Component A)	250 µL	200X concentrate in DMSO	• Room temperature • Desiccate • Protect from light	When stored as directed, kits are stable for 6 months.
Qubit™ ssDNA Buffer (Component B)	50 mL	Not applicable	• Room temperature	
Qubit™ ssDNA Standard #1 (Component C)	1 mL	0 ng/µL in TE buffer	• ≤4°C • Do not freeze	
Qubit™ ssDNA Standard #2 (Component D)	1 mL	20 ng/µL in TE buffer		

Introduction

The Qubit™ ssDNA Assay Kit for use with the Qubit® 2.0 Fluorometer makes DNA quantitation easy and accurate. The kit includes concentrated assay reagent, dilution buffer, and pre-diluted DNA standards. Simply dilute the reagent using the buffer provided, add your sample (any volume between 1 µL and 20 µL is acceptable), and read the concentration using the Qubit® 2.0 Fluorometer. The assay is accurate for initial sample concentrations from 50 pg/µL to 200 ng/µL providing an assay range of 1–200 ng. The assay is performed at room temperature, and the signal is stable for 30 minutes. Common contaminants such as salts, free nucleotides, solvents, detergents, or protein are well tolerated in the assay (*Appendix*, Table 2). In addition to the Qubit™ ssDNA Assay Kits described here, we also offer other kits for assaying RNA, protein, and dsDNA (*Appendix*, Table 3).

Note: All Qubit™ assay kits can also be used with the Qubit® 1.0 Fluorometer.

Before You Begin

Materials Required but Not Provided

- Plastic container (disposable) for mixing the Qubit™ working solution (step 1.3)
- Qubit® assay tubes (500 tubes, Cat. no. Q32856) or Axygen PCR-05-C tubes (VWR, part no. 10011-830)

Storing the Qubit™ ssDNA Assay Kit

The Qubit™ ssDNA reagent and buffer are designed for room temperature storage. The Qubit™ ssDNA reagent is supplied in DMSO, which freezes at temperatures lower than room temperature. Store the ssDNA standards at 4°C.

Critical Assay Parameters

Assay Temperature

The Qubit™ ssDNA assay for the Qubit® 2.0 Fluorometer delivers optimal performance when all solutions are at room temperature (22–28°C). The Qubit™ assays are designed to be performed at room temperature, as temperature fluctuations can influence the accuracy of the assay. To minimize temperature fluctuations, store the Qubit™ ssDNA reagent and the Qubit™ ssDNA buffer at room temperature and insert all assay tubes into the Qubit® 2.0 Fluorometer only for as much time as it takes for the instrument to measure the fluorescence, because the Qubit® 2.0 Fluorometer can raise the temperature of the assay solution significantly, even over a period of a few minutes. Do not hold the assay tubes in your hand before reading, as this warms the solution and results in a low reading.

Incubation Time

To allow the Qubit™ assay to reach optimal fluorescence, incubate the tubes for the DNA assays for 2 minutes after mixing the sample or standard with the working solution. After this incubation period, the fluorescence signal is stable for 30 minutes at room temperature.

Photobleaching of the Qubit™ Reagent

The Qubit™ reagents exhibit high photostability in the Qubit® 2.0 Fluorometer, showing <0.3% drop in fluorescence after 9 readings and <2.5% drop in fluorescence after 40 readings. It is important to remember, however, that if the assay tube remains in the Qubit® 2.0 Fluorometer for multiple readings, a temporary reduction in fluorescence is observed as the solution increases in temperature (see *Appendix*, Figure 2). Note that the temperature inside the Qubit® 2.0 Fluorometer may be as much as 3°C above room temperature after 1 hour. For this reason, if you want to perform multiple readings of a single tube, remove the tube from the instrument and let it equilibrate to room temperature for 30 seconds before taking another reading.

Calibrating the Qubit® 2.0 Fluorometer

For each assay, you have the choice to run a new calibration or to use the values from the previous calibration. As you first use the instrument, you should perform a new calibration each time. As you become familiar with the assays, the instrument, your pipetting accuracy, and significant temperature fluctuations within your laboratory, you should determine the level of comfort you have using the calibration data stored in from the last time the instrument was calibrated. Remember also that the fluorescence signal in the tubes containing standards and the samples is stable for no longer than 30 minutes. See Figure 1 in the *Appendix* for an example of the calibration curve used to generate the quantitation results.

Handling and Disposal

No data are currently available addressing the mutagenicity or toxicity of the Qubit™ ssDNA reagent (Component A). This reagent is known to bind nucleic acid and is provided as a solution in DMSO. Treat the Qubit™ ssDNA reagent with the same safety precautions as all other potential mutagens and dispose of the dye in accordance with local regulations.

Experimental Protocol

The protocol below assumes you are preparing standards for calibrating the Qubit® 2.0 Fluorometer. If you plan to use the last calibration performed on the instrument, you need fewer tubes (step 1.1) and less working solution (step 1.3). You can find more detailed instructions on the use of the Qubit® 2.0 Fluorometer (corresponding to steps 1.9–1.15 and 2.1–2.6, below) in the user manual accompanying the instrument.

1.1 Set up the required number of 0.5 mL tubes for standards and samples. The Qubit™ ssDNA assay requires 2 standards.

Note: Use only thin-wall, clear 0.5 mL PCR tubes. Acceptable tubes include Qubit® assay tubes (500 tubes, Invitrogen Cat. no. Q32856) or Axygen PCR-05-C tubes (VWR, part number 10011-830).

1.2 Label the tube lids.

1.3 Make the Qubit™ working solution by diluting the Qubit™ ssDNA reagent 1:200 in Qubit™ ssDNA buffer. Use a clean plastic tube each time you make Qubit™ working solution. **Do not mix the working solution in a glass container.**

Note: The final volume in each tube must be 200 µL. Each standard tube requires 190 µL of Qubit™ working solution, and each sample tube requires anywhere from 180 µL to 199 µL. Prepare sufficient Qubit™ working solution to accommodate all standards and samples.

For example, for 8 samples, prepare enough working solution for the samples and 2 standards: ~200 µL per tube in 10 tubes yields 2 mL of working solution (10 µL of Qubit™ reagent plus 1,990 µL of Qubit™ buffer).

1.4 Load 190 µL of Qubit™ working solution into each of the tubes used for standards.

1.5 Add 10 µL of each Qubit™ standard to the appropriate tube and mix by vortexing 2–3 seconds, being careful not to create bubbles.

Note: Careful pipetting is critical to ensure that exactly 10 µL of each Qubit™ ssDNA standard is added to 190 µL of Qubit™ working solution. It is also important to label the lid of each standard tube correctly as calibration of the Qubit® 2.0 Fluorometer requires that the standards are introduced to the instrument in the right order.

1.6 Load Qubit™ working solution into individual assay tubes so that the final volume in each tube after adding sample is 200 µL.

Note: Your sample can be anywhere between 1 µL and 20 µL, therefore, load each assay tube with a volume of Qubit™ working solution anywhere between 180 µL and 199 µL.

1.7 Add each of your samples to assay tubes containing the correct volume of Qubit™ working solution (prepared in step 1.6) and mix by vortexing 2–3 seconds. The final volume in each tube should be 200 µL.

1.8 Allow all tubes to incubate at room temperature for 2 minutes.

1.9 On the Home Screen of the Qubit® 2.0 Fluorometer, press **DNA**, and then select **ssDNA** as the assay type. The Standards Screen is automatically displayed.

Note: If you have already performed a calibration for the selected assay, Qubit® 2.0 Fluorometer will prompt you to choose between reading new standards and using the previous calibration. See *Calibrating the Qubit® Fluorometer* above for calibration guidelines.

1.10 On the Standards Screen, press **Yes** to run a new calibration or press **No** to use the last calibration.

1.11 If you pressed **No** on the Standards Screen, proceed to step 1.12. If you selected **Yes** to a run new calibration, follow instructions below.

Running a New Calibration

Insert the tube containing Standard #1 in the Qubit® 2.0 Fluorometer, close the lid, and press **Read**. The reading will take approximately 3 seconds.

Remove Standard #1.

Insert the tube containing Standard #2 in the Qubit® 2.0 Fluorometer, close the lid, and press **Read**.

Remove Standard #2.

1.12 If you pressed **No** on the Standards Screen, the Sample Screen will be automatically displayed. Insert a sample tube into the Qubit® 2.0 Fluorometer, close the lid, and press **Read**.

1.13 Upon the completion of the measurement, the result will be displayed on the screen.

Note: The value given by the Qubit® 2.0 Fluorometer at this stage corresponds to the concentration after your sample was diluted into the assay tube. You can record this value and perform the calculation yourself to find out the concentration of your original sample (see *Calculating the Concentration of Your Sample*, below) or the Qubit® 2.0 Fluorometer performs this calculation for you (see *Dilution Calculator*, next page).

1.14 To read the next sample, remove the sample from the Qubit® 2.0 Fluorometer, insert the next sample, and press **Read Next Sample**.

1.15 Repeat sample readings until all samples have been read.

Calculating the Concentration of Your Sample

The Qubit® 2.0 Fluorometer gives values for the Qubit™ ssDNA assay in ng/mL. This value corresponds to the concentration after your sample was diluted into the assay tube. To calculate the concentration of your sample, use the following equation:

$$\text{Concentration of your sample} = \text{QF value} \times \left(\frac{200}{x}\right)$$

where:

QF value = the value given by the Qubit® 2.0 Fluorometer

x = the number of microliters of sample you added to the assay tube

This equation generates a result with the same units as the value given by the Qubit® 2.0 Fluorometer (*i.e.*, if the Qubit® 2.0 Fluorometer gave a concentration in ng/mL, the result of the equation will be in ng/mL).

Dilution Calculator

The "Dilution Calculator" feature of the Qubit® 2.0 Fluorometer calculates the concentration of your original sample based on the volume of sample you have added to the assay tube. To have the Qubit® 2.0 Fluorometer perform this calculation for you, follow the instruction below.

2.1 Upon completion of the sample measurement, press **Calculate Stock Conc.** The Dilution Calculator Screen containing the volume roller wheel is displayed.

2.2 Using the volume roller wheel, select the volume of your original sample that you have added to the assay tube. When you stop scrolling, the Qubit® 2.0 Fluorometer calculates the original sample concentration based on the measured assay concentration.

2.3 To change the units in which the original sample concentration is displayed, press **ng/mL**. A pop-up window showing the current unit selection (as indicated by an adjacent red dash) opens.

2.4 Select the unit for your original sample concentration by touching the desired unit in the unit selection pop-up window. To close the unit selection pop-up window, touch anywhere on the screen outside the pop-up.

The Qubit® 2.0 Fluorometer automatically converts the units to your selection once the unit selection pop-up window is closed.

Note: The unit button next to your sample concentration reflects the change in the units (e.g., if you change the unit to pg/µL, the button will display pg/µL).

2.5 To save the data from your calculation to the Qubit® 2.0 Fluorometer, press **Save** on the Dilution Calculator screen. The last calculated value of your measurement will be saved in the .CSV file and tagged with a time and date stamp.

2.6 To exit the Dilution Calculator screen, press any navigator button on the bottom of the screen or **Read Next Sample**.

Note: When you navigate away from the Dilution Calculator screen, the Qubit® 2.0 Fluorometer saves the last values for the sample volume and the units in the Dilution Calculator screen only. Returning to the Dilution Calculator screen displays these last selected values.

Appendix

How the Qubit® 2.0 Fluorometer Calculates Concentrations

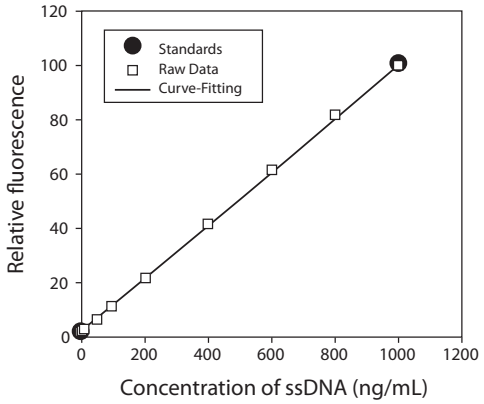

Figure 1. The curve-fitting algorithm used to determine concentration in the Qubit™ ssDNA assay. The Qubit® 2.0 Fluorometer generates concentration data based on the relationship between the two standards used in calibration. This plot shows the line corresponding to the curve-fitting algorithm (a modified Hill plot) used in the calculation of concentration data for the Qubit™ ssDNA assay. For reference, the positions of the standards and a set of data points from an actual experiment are shown superimposed onto the line, demonstrating that the curve-fitting algorithm gives accurate values for quantitation.

Contaminants Tolerated by the Qubit™ ssDNA Assay

Table 2. Effect of contaminants in the Qubit™ ssDNA assay.

Compound	Maximum Acceptable Concentration	% Signal Change*
Salts		
Ammonium acetate	50 mM	13% decrease
Sodium acetate	30 mM	3% decrease
Sodium chloride	100 mM	25% decrease
Zinc chloride	1 mM	43% decrease
Magnesium chloride	5 mM	34% decrease
Urea	2 M	47% increase
Organic Solvents		
Phenol	0.2%	19% decrease
Ethanol	10%	19% increase
Chloroform	2%	2% increase
Detergents		
Sodium dodecyl sulfate	0.01%	73% increase
Triton® X-100	0.1%	11% increase
Proteins		
Bovine serum albumin	2%	20% increase
IgG	0.1%	37% decrease
Other Compounds		
Polyethylene glycol	1%	29% increase
Agarose	0.1%	8% increase
ATP	0.1%	30% increase

*The compounds were incubated at the indicated concentrations with Qubit™ ssDNA assay in the presence of 660 ng/mL of a 24-mer M13 sequencing primer.

Qubit™ Assay Kits Compatible with the Qubit® 2.0 Fluorometer

A number of fluorescence-based quantitation kits are available for use with the Qubit® 2.0 Fluorometer. Table 3 will help you choose a kit based on the target molecule being measured and the number of assays you require.

Table 3. Qubit™ Assay Kits for use with the Qubit® 2.0 Fluorometer.

Product	Cat. no.	Number of Assays*	Target	Notes
Qubit™ dsDNA BR Assay Kit	Q32850	100	dsDNA	• core range (high confidence): 0.01 µg/mL to 5 µg/mL† • extended range (moderate confidence): 5 µg/mL to 10 µg/mL† • useful for quantitation of genomic and miniprep DNA samples • accurate in the presence of RNA, salts, solvents, proteins, and free nucleotides
Qubit™ dsDNA BR Assay Kit	Q32853	500		
Qubit™ dsDNA HS Assay Kit	Q32851	100	dsDNA	• core range (high confidence): 1 ng/mL to 500 ng/mL† • extended ranges (moderate confidence): 0.5 ng/mL to 1 ng/mL and 500 ng/mL to 600 ng/mL† • useful for quantitation of PCR products, viral DNA, and samples for subcloning • accurate in the presence of RNA, salts, solvents, proteins, and free nucleotides
Qubit™ dsDNA HS Assay Kit	Q32854	500		
Qubit™ ssDNA Assay Kit	Q10212	100	ssDNA	• core range (high confidence): 5 ng/mL to 1,000 ng/mL† • extended ranges (moderate confidence): 1 ng/mL to 5 ng/mL and 1,000 ng/mL to 1,200 ng/mL† • useful for quantitation of oligos, primers, denatured DNA, PCR products • accurate in the presence of salts, urea, solvents, proteins, ATP, and agarose
Qubit™ RNA Assay Kit	Q32852	100	RNA	• core range (high confidence): 25 ng/mL to 500 ng/mL† • extended ranges (moderate confidence): 20 ng/mL to 25 ng/mL and 500 ng/mL to 1,000 ng/mL† • useful for quantitation of samples for microarray, RT-PCR, and Northern blot procedures • accurate in the presence of DNA, salts, solvents, proteins, and free nucleotides
Qubit™ RNA Assay Kit	Q32855	500		
Qubit™ RNA BR Assay Kit	Q10210	100	RNA	• core range (high confidence): 0.1 µg/mL to 5 µg/mL† • extended ranges (moderate confidence): 0.05 µg/mL to 0.1 µg/mL and 5–6 µg/mL† • useful for quantitation of samples for microarray, RT-PCR, and Northern blot procedures • accurate in the presence of DNA, salts, solvents, proteins, and free nucleotides
Qubit™ RNA BR Assay Kit	Q10211	500		
Qubit™ Protein Assay Kit	Q33211	100	protein	• core range (high confidence): 1.25 µg/mL to 25 µg/mL† • extended ranges (moderate confidence): 1 µg/mL to 1.25 µg/mL and 25 µg/mL to 26 µg/mL† • little protein-to-protein difference in signal • accurate in the presence of DTT, β-mercaptoethanol, amino acids, and DNA • signal is stable for 3 hours
Qubit™ Protein Assay Kit	Q33212	500		

*Based on an assay volume of 200 µL. †Concentration ranges refer to the concentration of sample after dilution in the assay tube.

Product List **Current prices may be obtained from our website or from our Customer Service Department.**

Cat. no.	Product Name	Unit Size
Q10212	Qubit™ ssDNA Assay Kit, 100 assays *1–200 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Related products		
Q32850	Qubit™ dsDNA BR Assay Kit, 100 assays *2–1000 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q32853	Qubit™ dsDNA BR Assay Kit 500 assays *2–1000 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q32851	Qubit™ dsDNA HS Assay Kit, 100 assays *0.2–100 ng* *for use with the Qubit® 2.0 Fluorometer	1 kit
Q32854	Qubit™ dsDNA HS Assay Kit, 500 assays *0.2–100 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q10210	Qubit™ RNA BR Assay Kit, 100 assays *20–1000 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q10211	Qubit™ RNA BR Assay Kit, 500 assays *20–1000 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q32852	Qubit™ RNA Assay Kit, 100 assays *5–100 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q32855	Qubit™ RNA Assay Kit, 500 assays *5–100 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q33211	Qubit™ Protein Assay Kit, 100 assays *0.25–5 µg* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q33212	Qubit™ Protein Assay Kit, 500 assays *0.25–5 µg* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q32856	Qubit™ assay tubes *set of 500*	1 set
Q32866	Qubit® 2.0 Fluorometer	each
Q32867	Qubit® 2.0 Fluorometer USB	each
Q32868	Qubit® 2.0 Fluorometer International Power Cord (replacement)	each

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Qubit™ Protein Assay Kits

For use with the Qubit® 2.0 Fluorometer

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage*
Qubit™ protein reagent (Component A)	300 µL or 1.5 mL	200X concentrate in 1,2-propanediol	• Room temperature • Desiccate • Protect from light
Qubit™ protein buffer (Component B)	60 mL or 300 mL	Not applicable	• Room temperature
Qubit™ protein standard #1 (Component C)	1 mL or 5 mL	0 ng/µL in TE buffer with 2 mM sodium azide	• ≤4°C
Qubit™ protein standard #2 (Component D)	1 mL or 5 mL	200 ng/µL in TE buffer with 2 mM sodium azide	
Qubit™ protein standard #3 (Component E)	1 mL or 5 mL	400 ng/µL in TE buffer with 2 mM sodium azide	

* When stored as directed, kits are stable for 6 months.

Introduction

The Qubit™ Protein Assay Kits for use with the Qubit® 2.0 Fluorometer make protein quantitation easy and accurate. The kits provide concentrated assay reagent, dilution buffer, and pre-diluted BSA standards. Simply dilute the reagent using the buffer provided, add your sample (any volume between 1 µL and 20 µL is acceptable), and read the concentration using the Qubit® 2.0 Fluorometer. The assay is accurate for initial sample concentrations from 12.5 µg/mL to 5 mg/mL and exhibits low protein-to-protein variation (*Appendix*, Figure 1). The assay is performed at room temperature, and the signal is stable for 3 hours. Common contaminants, such as reducing reagents (DTT, β-mercaptoethanol), salts, free nucleotides, amino acids, solvents, or DNA, but not detergents, are well tolerated in the assay, although some very slight modifications for the procedure are required for other contaminants (*Appendix*, Table 2**).** In addition to the Qubit™ Protein Assay Kits described here, we also offer other kits for assaying dsDNA, and RNA (*Appendix*, Table 3).

Before You Begin

Materials Required but Not Provided

- Plastic container (disposable) for mixing the Qubit™ working solution (step 1.3)
- Qubit™ assay tubes (500 tubes, Cat. no. Q32856) or Axygen PCR-05-C tubes (VWR, part no. 10011-830)

Storing the Qubit™ Protein Assay Kits

The Qubit™ protein reagent and buffer are designed for room temperature storage. The Qubit™ protein reagent is supplied in DMSO, which freezes at temperatures lower than room temperature. Store the protein standards at 4˚C.

Critical Assay Parameters

Assay Temperature

The Qubit™ protein assay for the Qubit® 2.0 Fluorometer delivers optimal performance when all solutions are at room temperature (22–28°C). The Qubit™ assays is designed to be performed at room temperature, as temperature fluctuations can influence the accuracy of the assay (*Appendix*, Figure 2). To minimize temperature fluctuations, store the Qubit™ protein reagent and the Qubit™ protein buffer at room temperature and insert all assay tubes into the Qubit® 2.0 Fluorometer only for as much time as it takes for the instrument to measure the fluorescence, because the Qubit® 2.0 Fluorometer can raise the temperature of the assay solution significantly, even over a period of a few minutes. Do not hold the assay tubes in your hand before reading, as this will warm the solution and result in a low reading.

Incubation Time

To allow the Qubit™ protein assay to reach optimal fluorescence, incubate the tubes for 15 minutes after mixing the sample or standard with the working solution. After this incubation period, the fluorescence signal is stable for 3 hours at room temperature. For greatest accuracy of the protein assay, the incubation time of the samples should be within 10 minutes of the incubation time of the standards.

Photobleaching of the Qubit™ Reagent

The Qubit™ reagents exhibit high photostability in the Qubit® 2.0 Fluorometer, showing <0.3% drop in fluorescence after 9 readings and <2.5% drop in fluorescence after 40 readings. It is important to remember, however, that if the assay tube remains in the Qubit® 2.0 Fluorometer for multiple readings, a temporary reduction in fluorescence will be observed as the solution increases in temperature (see *Appendix*, Figure 2). (The temperature inside the Qubit® 2.0 Fluorometer may be as much as 3°C above room temperature after 1 hour.) For this reason, if you want to perform multiple readings of a single tube, you should remove the tube from the instrument and let it equilibrate to room temperature for 30 seconds before taking another reading.

Calibrating the Qubit® 2.0 Fluorometer

For each assay, you have the choice to run a new calibration or to use the values from the previous calibration. As you first use the instrument, perform a new calibration each time. As you become familiar with the assays, the instrument, your pipetting accuracy, and significant temperature fluctuations within your laboratory, you should determine the level of comfort you have using the calibration data stored in from the last time the instrument was calibrated. Remember also that the fluorescence signal in the tubes containing standards and the samples is stable for no longer than 3 hours. See Figure 3 in the *Appendix* for an example of the calibration curve used to generate the quantitation results.

Handling and Disposal

No data are available addressing the mutagenicity or toxicity of the Qubit™ protein reagent (Component A). This reagent is an organic dye and is provided as a solution in 1,2-propanediol. Treat the Qubit™ protein reagent with the same safety precautions as other materials with similar properties and dispose of the dye in accordance with local regulations.

Experimental Protocol

The protocol below assumes you are preparing standards for calibrating the Qubit® 2.0 Fluorometer. If you plan to use the last calibration performed on the instrument, you need fewer tubes (step 1.1) and less working solution (step 1.3). More detailed instructions on the use of the Qubit® 2.0 Fluorometer (corresponding to steps 1.9–1.15 and 2.1–2.6) can be found in the user manual accompanying the instrument.

1.1 Set up the number of 0.5 mL tubes you need for standards and samples. The Qubit™ protein assay requires **3 standards**.

Note: Use only thin-wall, clear 0.5 mL optical-grade real-time PCR tubes. Acceptable tubes include Qubit® assay tubes (500 tubes, Cat. no. Q32856) or Axygen PCR-05-C tubes (VWR, part no. 10011-830).

1.2 Label the tube lids.

1.3 Make the Qubit™ working solution by diluting the Qubit™ protein reagent 1:200 in Qubit™ protein buffer. Use a clean plastic tube each time you make Qubit™ working solution. Do not mix the working solution in a glass container.

Note: The final volume in each tube must be 200 µL. Each standard tube requires 190 µL of Qubit™ working solution, and each sample tube requires anywhere from 180 µL to 199 µL. Prepare sufficient Qubit™ working solution to accommodate all standards and samples. For example, for 7 samples, prepare enough working solution for the samples and 3 standards: ~200 µL per tube in 10 tubes yields 2 mL of working solution (10 µL of Qubit™ reagent plus 1,990 µL of Qubit™ buffer).

1.4 Load 190 µL of Qubit™ working solution into each of the tubes used for standards.

1.5 Add 10 µL of each Qubit™ standard to the appropriate tube and mix by vortexing 2–3 seconds, being careful not to create bubbles.

Note: Careful pipetting is critical to ensure that exactly 10 µL of each Qubit™ protein standard is added to 190 µL of Qubit™ working solution. It is also important to label the lid of each standard tube correctly as calibration of the Qubit® 2.0 Fluorometer requires that the standards be introduced to the instrument in the right order.

1.6 Load Qubit™ working solution into individual assay tubes so that the final volume in each tube after adding sample is 200 µL.

Note: Your sample can be anywhere between 1 µL and 20 µL, therefore, load each assay tube with a volume of Qubit™ working solution anywhere between 180 µL and 199 µL.

1.7 Add each of your samples to assay tubes containing the correct volume of Qubit™ working solution (prepared in step 1.6) and mix by vortexing 2–3 seconds. The final volume in each tube should be 200 µL.

1.8 Allow all tubes to incubate at room temperature for 15 minutes.

1.9 On the Home Screen of the Qubit® 2.0 Fluorometer, press **Protein**. The Standards Screen is automatically displayed.

Note: If you have already performed a calibration for the selected assay, Qubit® 2.0 Fluorometer will prompt you to choose between reading new standards and using the previous calibration. See *Calibrating the Qubit® 2.0 Fluorometer* above for calibration guidelines.

1.10 On the Standards Screen, press **Yes** to run a new calibration or press **No** to use the last calibration.

1.11 If you pressed **No** on the Standards Screen, proceed to step 1.12. If you selected **Yes** to a run new calibration, follow instructions below.

Running a New Calibration

Insert the tube containing Standard #1 in the Qubit® 2.0 Fluorometer, close the lid, and press **Read**. The reading will take approximately 3 seconds.

Remove Standard #1.

Insert the tube containing Standard #2 in the Qubit® 2.0 Fluorometer, close the lid, and press **Read**.

Remove Standard #2.

Insert the tube containing Standard #3 in the Qubit® 2.0 Fluorometer, close the lid, and press **Read**.

Remove Standard #3.

1.12 If you pressed **No** on the Standards Screen, the Sample Screen will be automatically displayed. Insert a sample tube into the Qubit® 2.0 Fluorometer, close the lid, and press **Read**.

1.13 Upon the completion of the measurement, the result will be displayed on the screen.

Note: The value given by the Qubit® 2.0 Fluorometer at this stage corresponds to the concentration after your sample was diluted into the assay tube. You can record this value and perform the calculation yourself to find out the concentration of your original sample (see *Calculating the Concentration of Your Sample*, below) or the Qubit® 2.0 Fluorometer performs this calculation for you (see *Dilution Calculator*, next page).

1.14 To read the next sample, remove the sample from the Qubit® 2.0 Fluorometer, insert the next sample, and press **Read Next Sample**.

1.15 Repeat sample readings until all samples have been read.

Calculating the Concentration of Your Sample

The Qubit® 2.0 Fluorometer gives values for the Qubit™ protein assay in µg/mL. This value corresponds to the concentration after your sample was diluted into the assay tube. To calculate the concentration of your sample, use the following equation:

$$\text{Concentration of your sample} = \text{QF value} \times \left(\frac{200}{x}\right)$$

where:

QF value = the value given by the Qubit® 2.0 Fluorometer

x = the number of microliters of sample you added to the assay tube

This equation generates a result with the same units as the value given by the Qubit® 2.0 Fluorometer (i.e., if the Qubit® 2.0 Fluorometer gave a concentration in µg/mL, the result of the equation will be in µg/mL).

Dilution Calculator

2.1 Upon completion of the sample measurement, press **Calculate Stock Conc.** The Dilution Calculator Screen containing the volume roller wheel is displayed.

2.2 Using the volume roller wheel, select the volume of your original sample that you have added to the assay tube. When you stop scrolling, the Qubit® 2.0 Fluorometer calculates the original sample concentration based on the measured assay concentration.

2.3 To change the units in which the original sample concentration is displayed, press **µg/mL**. A pop-up window showing the current unit selection (indicated by an adjacent red dash) opens.

2.4 Select the unit for your original sample concentration by touching the desired unit in the unit selection pop-up window. To close the unit selection pop-up window, touch anywhere on the screen outside the pop-up. The Qubit® 2.0 Fluorometer automatically converts the units to your selection once the unit selection pop-up window is closed.

Note: The unit button next to your sample concentration reflects the change in the units (e.g., if you change the unit to pg/µL, the button will display pg/µL).

2.5 To save the data from your calculation to the Qubit® 2.0 Fluorometer, press **Save** on the Dilution Calculator screen. The last calculated value of your measurement will be saved as a .CSV file and tagged with a time and date stamp.

2.6 To exit the Dilution Calculator screen, press any navigator button on the bottom of the screen or **Read Next Sample**.

Note: When you navigate away from the Dilution Calculator screen, the Qubit® 2.0 Fluorometer saves the last values for the sample volume and the units in the Dilution Calculator screen only. Returning to the Dilution Calculator screen displays these last selected values.

Appendix

Protein-to-Protein Variation of the Qubit™ Protein Assay

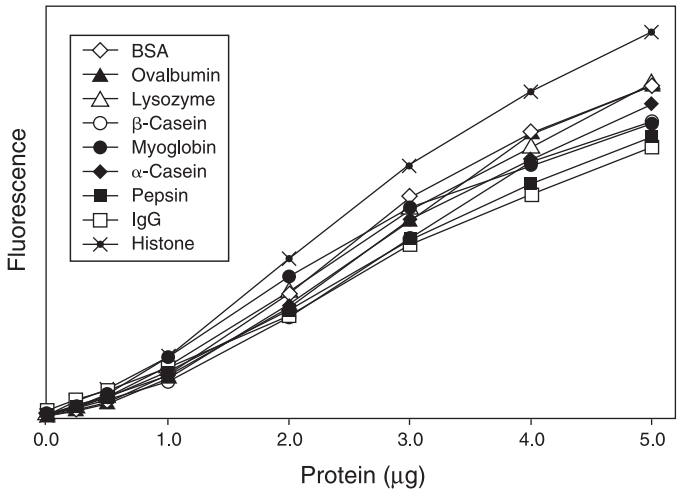

Figure 1. Low protein-to-protein variation in the Qubit™ protein assay (Q33211, Q33212). Solutions of the following proteins were prepared, diluted, and assayed in the Qubit™ protein assay: bovine serum albumin (BSA), chicken-egg ovalbumin, chicken-egg lysozyme, bovine-milk β-casein, equine myoglobin, bovine-milk α-casein, porcine pepsin, mouse immunoglobulin (IgG), and calf-thymus histone. Fluorescence was measured at 485/590 nm and plotted versus the mass of the protein sample. At 3 µg, the fluorescence variation was 12.4%, or 8.7% excluding the basic histone protein. Background fluorescence has not been subtracted.

Effect of Temperature on the Qubit™ Protein Assay

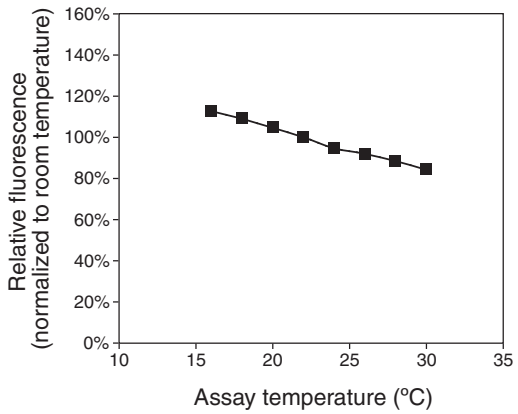

Figure 2. Plot of fluorescence vs. temperature for the Qubit™ protein assay. The Qubit™ assays were designed to be performed at room temperature, as temperature fluctuations can influence the accuracy of the assay.

How the Qubit® 2.0 Fluorometer Calculates Concentration

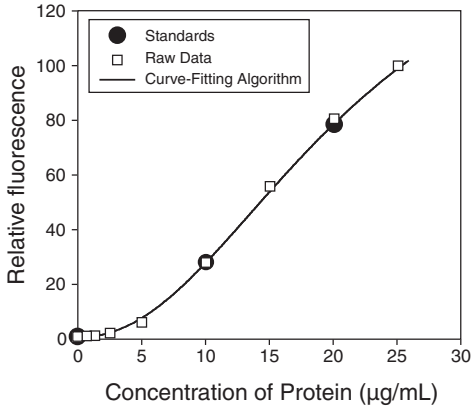

Figure 3. The curve-fitting algorithm used to determine concentration in the Qubit™ protein assay. The Qubit® 2.0 Fluorometer generates concentration data based on the relationship between the two standards used in calibration. This plot shows the line corresponding to the curve-fitting algorithm (a modified Hill plot) used in the calculation of concentration data for the Qubit™ protein assay. For reference, the positions of the standards and a set of data points from an actual experiment are shown superimposed onto the line, demonstrating that the curve-fitting algorithm gives accurate values for quantitation.

Contaminants Tolerated by the Qubit™ Protein Assay

Table 2. Effect of contaminants in the Qubit™ protein assay, tested over the range 1.25 µg/mL to 25 µg/mL.*

Contaminant	Final Concentration in the Assay	Concentration in 20 µL Sample	Concentration in 10 µL Sample	Result
Sodium chloride	20 mM	200 mM	400 mM	OK †
Magnesium chloride	2 mM	20 mM	40 mM	OK
Potassium chloride ‡	200 mM	200 mM	400 mM	OK
Calcium chloride ‡	2 mM	20 mM	40 mM	OK
Ammonium sulfate	5 mM	50 mM	100 mM	OK †
DTT	1 mM	10 mM	20 mM	OK †
β-Mercaptoethanol	1 mM	10 mM	20 mM	OK
EDTA	1 mM	10 mM	20 mM	OK
Sodium azide	1 mM	10 mM	20 mM	OK
HEPES, pH 7.4	5 mM	50 mM	100 mM	OK
Potassium phosphate, pH 7.4	5 mM	50 mM	100 mM	OK
PBS, pH 7.4	1 mM KPO_4 15 mM NaCl	10 mM KPO_4 150 mM NaCl	20 mM KPO_4 300 mM NaCl	Protocol modification required ***
Sucrose	50 mM	500 mM	1 M	OK
Sucrose	100 mM	1 M	2 M	NR
Glycerol	1%	10%	20%	OK †
Imidazole	1.25 mM	12.5 mM	25 mM	OK
SDS	0.01%	0.1%	0.2%	OK †
SDS	0.02%	0.2%	0.4%	NR
Tween 20	0.001%	0.01%	0.02%	NR
Triton® X-100	0.001%	0.01%	0.02%	NR
Amino acids §	100 µg/mL:	1 mg/mL:	2 mg/mL:	OK
dNTPs **	100 µM	1 mM	2 mM	OK †
DNA	5 µg/mL	50 µg/mL	100 µg/mL	OK †
DNA	10% ††	10% ††	10% ††	OK
DNA	50% ††	50% ††	50% ††	NR

***** BSA standards were assayed in the presence or absence of contaminants at the indicated final concentrations. Equivalent concentrations (approximate) in 20 µL or 10 µL sample volumes are also listed. Results are given as OK, usually less than 10% perturbation, or as NR (not recommended). **†** An acceptable result, but with some distortion of the standard curve. For best results, add the same amount of contaminant to the standard samples. **‡** A precipitate was observed. **§** A mixture of 19 amino acids. ****** A mixture of dATP, dCTP, dGTP, and dTTP. **††** For each data point, the DNA mass was a fixed percentage of the protein mass. *** For accurate results, add the same amount of PBS to standard samples.

Qubit™ Assay Kits Compatible with the Qubit® 2.0 Fluorometer

A number of fluorescence-based quantitation kits are available for use with the Qubit® 2.0 Fluorometer. Table 3 will help you choose a kit based on the target molecule being measured and the number of assays you require.

Table 3. Qubit™ Assay Kits for use with the Qubit® 2.0 Fluorometer.

Product	Cat. no.	Number of Assays*	Target	Notes
Qubit™ dsDNA BR Assay Kit	Q32850	100	dsDNA	• core range (high confidence): 0.01 µg/mL to 5 µg/mL† • extended range (moderate confidence): 5 µg/mL to 10 µg/mL† • useful for quantitation of genomic and miniprep DNA samples • accurate in the presence of RNA, salts, solvents, proteins, and free nucleotides
Qubit™ dsDNA BR Assay Kit	Q32853	500		
Qubit™ dsDNA HS Assay Kit	Q32851	100	dsDNA	• core range (high confidence): 1 ng/mL to 500 ng/mL† • extended ranges (moderate confidence): 0.5 ng/mL to 1 ng/mL and 500 ng/mL to 600 ng/mL† • useful for quantitation of PCR products, viral DNA, and samples for subcloning • accurate in the presence of RNA, salts, solvents, proteins, and free nucleotides
Qubit™ dsDNA HS Assay Kit	Q32854	500		
Qubit™ ssDNA Assay Kit	Q10212	100	ssDNA	• core range (high confidence): 5 ng/mL to 1,000 ng/mL† • extended ranges (moderate confidence): 1 ng/mL to 5 ng/mL and 1,000 ng/mL to 1,200 ng/mL† • useful for quantitation of oligos, primers, denatured DNA, PCR products • accurate in the presence of salts, urea, solvents, proteins, ATP, and agarose
Qubit™ RNA Assay Kit	Q32852	100	RNA	• core range (high confidence): 25 ng/mL to 500 ng/mL† • extended ranges (moderate confidence): 20 ng/mL to 25 ng/mL and 500 ng/mL to 1,000 ng/mL† • useful for quantitation of samples for microarray, RT-PCR, and Northern blot procedures • accurate in the presence of DNA, salts, solvents, proteins, and free nucleotides
Qubit™ RNA Assay Kit	Q32855	500		
Qubit™ RNA BR Assay Kit	Q10210	100	RNA	• core range (high confidence): 0.1 µg/mL to 5 µg/mL† • extended ranges (moderate confidence): 0.05 µg/mL to 0.1 µg/mL and 5–6 µg/mL† • useful for quantitation of samples for microarray, RT-PCR, and Northern blot procedures • accurate in the presence of DNA, salts, solvents, proteins, and free nucleotides
Qubit™ RNA BR Assay Kit	Q10211	500		
Qubit™ Protein Assay Kit	Q33211	100	protein	• core range (high confidence): 1.25 µg/mL to 25 µg/mL† • extended ranges (moderate confidence): 1 µg/mL to 1.25 µg/mL and 25 µg/mL to 26 µg/mL† • little protein-to-protein difference in signal • accurate in the presence of DTT, β-mercaptoethanol, amino acids, and DNA • signal is stable for 3 hours
Qubit™ Protein Assay Kit	Q33212	500		

*Based on an assay volume of 200 µL. †Concentration ranges refer to the concentration of sample after dilution in the assay tube.

Product List **Current prices may be obtained from our website or from our Customer Service Department.**

Cat. no.	Product Name	Unit Size
Q33211	Qubit™ Protein Assay Kit, 100 assays *0.25–5 µg* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q33212	Qubit™ Protein Assay Kit, 500 assays *0.25–5 µg* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Related products		
Q10210	Qubit™ RNA BR Assay Kit, 100 assays *20–1000 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q10211	Qubit™ RNA BR Assay Kit, 500 assays *20–1000 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q32852	Qubit™ RNA Assay Kit, 100 assays *5–100 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q32855	Qubit™ RNA Assay Kit, 500 assays *5–100 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q10212	Qubit™ ssDNA Assay Kit, 100 assays *1–200 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q32850	Qubit™ dsDNA BR Assay Kit, 100 assays *2–1000 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q32853	Qubit™ dsDNA BR Assay Kit 500 assays *2–1000 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q32851	Qubit™ dsDNA HS Assay Kit, 100 assays *0.2–100 ng* *for use with the Qubit® 2.0 Fluorometer	1 kit
Q32854	Qubit™ dsDNA HS Assay Kit, 500 assays *0.2–100 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q32856	Qubit™ assay tubes *set of 500*	1 set
Q32866	Qubit® 2.0 Fluorometer	each
Q32867	Qubit® 2.0 Fluorometer USB	each
Q32868	Qubit® 2.0 Fluorometer International Power Cord (replacement)	each

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Qubit™ RNA Assay Kits

For use with the Qubit® 2.0 Fluorometer

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage	Stability
Qubit™ RNA Assay Kit, 100 assays (Q32852)				
Qubit™ RNA Reagent (Component A)	250 µL	200X concentrate in DMSO	• Room temperature • Desiccate • Protect from light	When stored as directed, kits are stable for 6 months.
Qubit™ RNA Buffer (Component B)	50 mL	NA	• Room temperature	
Qubit™ RNA Standard #1 (Component C)	1 tube containing 1 mL	0 ng/µL in TE buffer	• ≤4°C	
Qubit™ RNA Standard #2 (Component D)	4 tubes each containing 250 µL	10 ng/µL in TE buffer		
Qubit™ RNA Assay Kit, 500 assays (Q32855)				
Qubit™ RNA Reagent (Component A)	1.25 mL	200X concentrate in DMSO	• Room temperature • Desiccate • Protect from light	When stored as directed, kits are stable for 6 months.
Qubit™ RNA Buffer (Component B)	250 mL	NA	• Room temperature	
Qubit™ RNA Standard #1 (Component C)	1 tube containing 5 mL	0 ng/µL in TE buffer	• ≤4°C	
Qubit™ RNA Standard #2 (Component D)	10 tubes each containing 500 µL	10 ng/µL in TE buffer		

NA = Not applicable.

Introduction

The Qubit™ RNA Assay Kits for use with the Qubit® 2.0 Fluorometer make RNA quantitation easy and accurate. The kit provides concentrated assay reagent, dilution buffer, and prediluted RNA standards. Simply dilute the reagent using the buffer provided, add your sample (any volume between 1 µL and 20 µL is acceptable), and read the concentration using the Qubit® 2.0 Fluorometer. The assay is highly selective for RNA over double-stranded DNA (dsDNA) (*Appendix*, Figure 1) and is accurate for initial sample concentrations from 250 pg/µL to 100 ng/µL. The assay is performed at room temperature, and the signal is stable for 3 hours. Common contaminants, such as salts, free nucleotides, solvents, detergents, or protein are well tolerated in the assay (*Appendix*, Table 2). In addition to the Qubit™ RNA Assay Kits described here, we also offer other kits for assaying DNA and protein (*Appendix*, Table 3).

To determine the purity of your sample, use the Qubit™ RNA Assay Kit together with the Qubit™ dsDNA BR Assay Kit. Thease measurements will give you a much better indication of sample purity than that produced by an A_{260}/A_{280} measurement. To measure protein contamination in nucleic acid samples, simply run 1–20 µL of the sample in the Qubit™ protein assay.

Note: All Qubit™ assay kits can also be used with the Qubit® 1.0 Fluorometer.

Before You Begin

Materials Required but Not Provided

- Plastic container (disposable) for mixing the Qubit™ working solution (step 1.3)
- Qubit™ assay tubes (500 tubes, Cat. no. Q32856) or Axygen PCR-05-C tubes (VWR, part no. 10011-830)

Storing the Qubit™ RNA Assay Kits

The Qubit™ RNA reagent and buffer are designed for room temperature storage. The Qubit™ RNA reagent is supplied in DMSO, which freezes at temperatures lower than room temperature. Store the RNA standards at 4°C.

The Qubit™ RNA reagent is sensitive to light. Store the vial in the dark when not in use.

Critical Assay Parameters

Assay Temperature

The Qubit™ RNA assay for the Qubit® 2.0 Fluorometer delivers optimal performance when all solutions are at room temperature (22–28˚C). The Qubit™ assays were designed to be performed at room temperature, as temperature fluctuations can influence the accuracy of the assay (*Appendix*, Figure 2). To minimize temperature fluctuations, store the Qubit™ RNA reagent and the Qubit™ RNA buffer at room temperature and insert all assay tubes into the Qubit® 2.0 Fluorometer only for as much time as it takes for the instrument to measure the fluorescence, because the Qubit® 2.0 Fluorometer can raise the temperature of the assay solution significantly, even over a period of a few minutes. Do not hold the assay tubes in your hand before reading, as this will warm the solution and result in a low reading.

Incubation Time

To allow the Qubit™ assay to reach optimal fluorescence, incubate the tubes for the DNA and RNA assays for 2 minutes after mixing the sample or standard with the working solution. After this incubation period, the fluorescence signal is stable for 3 hours at room temperature.

Photobleaching of the Qubit™ Reagent

The Qubit™ reagents exhibit high photostability in the Qubit® 2.0 Fluorometer, showing <0.3% drop in fluorescence after 9 readings and <2.5% drop in fluorescence after 40 readings. It is important to remember, however, that if the assay tube remains in the Qubit® 2.0 Fluorometer for multiple readings, a temporary reduction in fluorescence will be observed as the solution increases in temperature (see *Appendix*, Figure 2). (The temperature inside the Qubit® 2.0 Fluorometer may be as much as 3˚C above room temperature after 1 hour.) For this reason, if you want to perform multiple readings of a single tube, you should remove the tube from the instrument and let it equilibrate to room temperature for 30 seconds before taking another reading.

Calibrating the Qubit® 2.0 Fluorometer

For each assay, you have the choice to run a new calibration or to use the values from the previous calibration. As you first use the instrument, you should perform a new calibration each time. As you become familiar with the assays, the instrument, your pipetting accuracy, and significant temperature fluctuations within your laboratory, you should determine the level of comfort you have using the calibration data stored in from the last time the instrument was calibrated. Remember also that the fluorescence signal in the tubes containing standards and the samples is stable for no longer than 3 hours. See Figure 3 in the *Appendix* for an example of the calibration curve used to generate the quantitation results.

Handling and Disposal

We must caution that no data are available addressing the mutagenicity or toxicity of the Qubit™ RNA reagent (Component A). This reagent is known to bind nucleic acid and is provided as a solution in DMSO. Treat the Qubit™ RNA reagent with the same safety precautions as all other potential mutagens and dispose of the dye in accordance with local regulations.

The calibration standards included in the Qubit™ RNA Assay Kit are high-quality rRNA standards. The integrity and concentration of these standards is critical to the optimal performance of the Qubit™ RNA assay. As such, we highly recommend treating the rRNA standards as you would any other precious RNA. Use appropriate RNAse-free handling techniques, including RNAse-free gloves, pipette tips, and tubes. Keep the tube lids closed whenever possible; do not touch the pipet to the inside wall of the tube when withdrawing a sample, and return the rRNA standard to the refrigerator as soon as possible after use.

Experimental Protocol

Performing the Qubit™ RNA Assay

The protocol below assumes you will be preparing standards for calibrating the Qubit® 2.0 Fluorometer. If you plan to use the last calibration performed on the instrument, you will need fewer tubes (step 1.1) and less working solution (step 1.3). More detailed instructions on the use of the Qubit® 2.0 Fluorometer (corresponding to numbered steps 1.9–1.16, below) can be found in the user manual accompanying that instrument. For sample purity determinations, it is possible to use the Qubit® 2.0 Fluorometer to calculate the amount of dsDNA and RNA in the same sample. Simply perform each assay for your sample.

1.1 Set up the number of 0.5 mL tubes you will need for standards and samples. The Qubit™ RNA assay requires 2 standards.

Note: Use only thin-wall, clear 0.5 mL optical-grade real-time PCR tubes. Acceptable tubes include Qubit™ assay tubes (500 tubes, Invitrogen Cat. no. Q32856) or Axygen PCR-05-C tubes (VWR, part number 10011-830).

1.2 Label the tube lids.

1.3 Make the Qubit™ working solution by diluting the Qubit™ RNA reagent 1:200 in Qubit™ RNA buffer. Use a clean plastic tube each time you make Qubit™ working solution. Do not mix the working solution in a glass container.

Note: The final volume in each tube must be 200 µL. Each standard tube will require 190 µL of Qubit™ working solution, and each sample tube will require anywhere from 180 µL to 199 µL. Prepare sufficient Qubit™ working solution to accommodate all standards and samples. For example, for 8 samples, prepare enough working solution for the samples and 2 standards: ~200 µL per tube in 10 tubes yields 2 mL of working solution (10 µL of Qubit™ reagent plus 1,990 µL of Qubit™ buffer).

1.4 Load 190 µL of Qubit™ working solution into each of the tubes used for standards.

1.5 Add 10 µL of each Qubit™ standard to the appropriate tube and mix by vortexing 2–3 seconds, being careful not to create bubbles..

Note: Careful pipetting is critical to ensure that exactly 10 µL of each Qubit™ RNA standard is added to 190 µL of Qubit™ working solution. It is also important to label the lid of each standard tube correctly as calibration of the Qubit® 2.0 Fluorometer requires that the standards be introduced to the instrument in the right order.

1.6 Load Qubit™ working solution into individual assay tubes so that the final volume in each tube after adding sample is 200 µL.

Note: Your sample can be anywhere between 1 µL and 20 µL, therefore, load each assay tube with a volume of Qubit™ working solution anywhere between 180 µL and 199 µL.

1.7 Add each of your samples to assay tubes containing the correct volume of Qubit™ working solution (prepared in step 1.6) and mix by vortexing 2–3 seconds. The final volume in each tube should be 200 µL.

1.8 Allow all tubes to incubate at room temperature for 2 minutes.

1.9 On the Home Screen of the Qubit® 2.0 Fluorometer, press **RNA**, and then select **RNA** again as the assay type. The Standards Screen is automatically displayed.

Note: If you have already performed a calibration for the selected assay, Qubit® 2.0 Fluorometer will prompt you to choose between reading new standards and using the previous calibration. See *Calibrating the Qubit® 2.0 Fluorometer* above for calibration guidelines.

1.10 On the Standards Screen, press **Yes** to run a new calibration or press **No** to use the last calibration.

1.11 If you pressed **No** on the Standards Screen, proceed to step 1.12. If you selected **Yes** to a run new calibration, follow instructions below.

Running a New Calibration

Insert the tube containing Standard #1 in the Qubit® 2.0 Fluorometer, close the lid, and press **Read**. The reading will take approximately 3 seconds.
Remove Standard #1.
Insert the tube containing Standard #2 in the Qubit® 2.0 Fluorometer, close the lid, and press **Read**.
Remove Standard #2.

1.12 If you pressed **No** on the Standards Screen, the Sample Screen will be automatically displayed. Insert a sample tube into the Qubit® 2.0 Fluorometer, close the lid, and press **Read**.

1.13 Upon the completion of the measurement, the result will be displayed on the screen.

Note: The value given by the Qubit® 2.0 Fluorometer at this stage corresponds to the concentration after your sample was diluted into the assay tube. You can record this value and perform the calculation yourself to find out the concentration of your original sample (see *Calculating the Concentration of Your Sample*, next page) or the Qubit® 2.0 Fluorometer performs this calculation for you (see *Dilution Calculator*, next page).

1.14 To read the next sample, remove the sample from the Qubit® 2.0 Fluorometer, insert the next sample, and press **Read Next Sample**.

1.15 Repeat sample readings until all samples have been read.

Calculating the Concentration of Your Sample

The Qubit® 2.0 Fluorometer gives values for the Qubit™ RNA assay in ng/mL. This value corresponds to the concentration after your sample was diluted into the assay tube. To calculate the concentration of your sample, use the following equation:

$$\text{Concentration of your sample} = \text{QF value} \times \left(\frac{200}{x}\right)$$

where:
QF value = the value given by the Qubit® 2.0 Fluorometer
x = the number of microliters of sample you added to the assay tube

This equation generates a result with the same units as the value given by the Qubit® 2.0 Fluorometer (i.e., if the Qubit® 2.0 Fluorometer gave a concentration in ng/mL, the result of the equation will be in ng/mL).

Dilution Calculator

The "Dilution Calculator" feature of the Qubit® 2.0 Fluorometer calculates the concentration of your original sample based on the volume of sample you have added to the assay tube. To have the Qubit® 2.0 Fluorometer perform this calculation for you, follow the instruction below.

2.1 Upon completion of the sample measurement, press **Calculate Stock Conc.** The Dilution Calculator Screen containing the volume roller wheel is displayed.

2.2 Using the volume roller wheel, select the volume of your original sample that you have added to the assay tube. When you stop scrolling, the Qubit® 2.0 Fluorometer calculates the original sample concentration based on the measured assay concentration.

2.3 To change the units in which the original sample concentration is displayed, press **ng/mL**. A pop-up window showing the current unit selection (as indicated by an adjacent red dash) opens.

2.4 Select the unit for your original sample concentration by touching the desired unit in the unit selection pop-up window. To close the unit selection pop-up window, touch anywhere on the screen outside the pop-up.

The Qubit® 2.0 Fluorometer automatically converts the units to your selection once the unit selection pop-up window is closed.

Note: The unit button next to your sample concentration reflects the change in the units (e.g., if you change the unit to pg/µL, the button will display pg/µL).

2.5 To save the data from your calculation to the Qubit® 2.0 Fluorometer, press **Save** on the Dilution Calculator screen. The last calculated value of your measurement will be saved as a .CSV file and tagged with a time and date stamp.

2.6 To exit the Dilution Calculator screen, press any navigator button on the bottom of the screen or **Read Next Sample**.

Note: When you navigate away from the Dilution Calculator screen, the Qubit® 2.0 Fluorometer saves the last values for the sample volume and the units in the Dilution Calculator screen only. Returning to the Dilution Calculator screen displays these last selected values.

Appendix

Selectivity of the Qubit™ RNA Assay

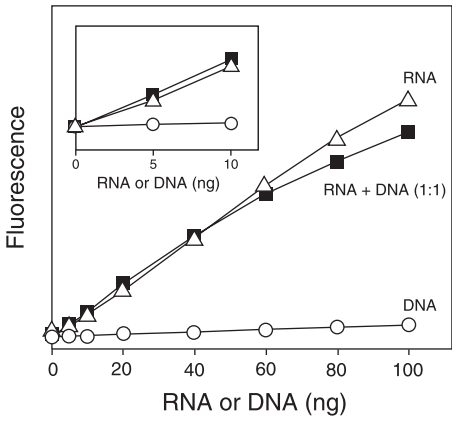

Figure 1. RNA selectivity and sensitivity of the Qubit™ RNA assay (Q32852, Q32855). Triplicate 10 µL samples of *E. coli* rRNA (△), λ DNA (○), or a 1:1 mixture of RNA and DNA (■) were assayed in the Qubit™ RNA assay. Fluorescence was measured at 630/680 nm and plotted versus the mass of nucleic acid for the RNA alone or DNA alone, or versus the mass of the RNA component in the 1:1 mixture. The variation (CV) of replicate RNA determinations was ≤10%. The inset is an enlargement of the graph to show the sensitivity of the assay for RNA. Background fluorescence has not been subtracted.

Effect of Temperature on the Qubit™ RNA Assay

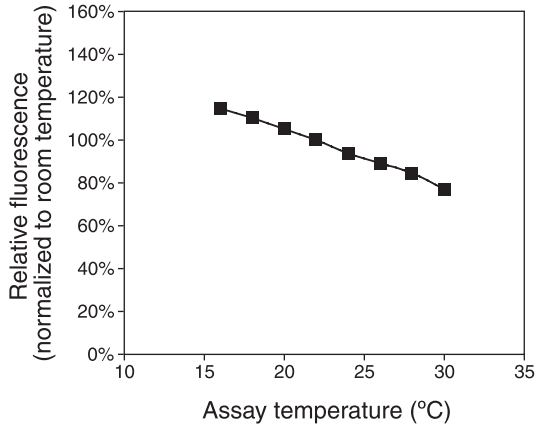

Figure 2. Plot of fluorescence vs. temperature for the Qubit™ RNA assay. The Qubit™ assays were designed to be performed at room temperature, as temperature fluctuations can influence the accuracy of the assay.

How the Qubit® 2.0 Fluorometer Calculates Concentration

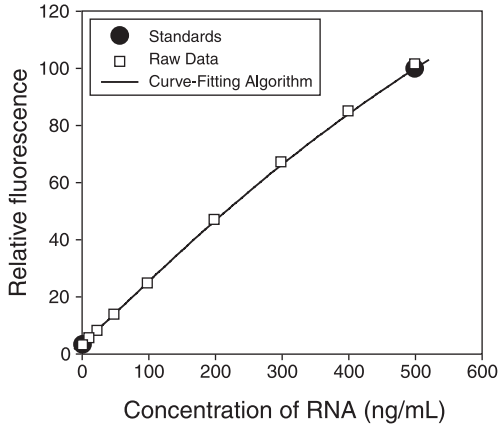

Figure 3. The curve-fitting algorithm used to determine concentration in the Qubit™ RNA assay. The Qubit® 2.0 Fluorometer generates concentration data based on the relationship between the two standards used in calibration. This plot shows the line corresponding to the curve-fitting algorithm (a modified Hill plot) used in the calculation of concentration data for the Qubit™ RNA assay. For reference, the positions of the standards and a set of data points from an actual experiment are shown superimposed onto the line, demonstrating that the curve-fitting algorithm gives accurate values for quantitation.

Contaminants Tolerated by the Qubit™ RNA Assay

Table 2. Effect of contaminants in the Qubit™ RNA assay, tested over the range 25 ng/mL to 500 ng/mL.*

Contaminant	Final Concentration in the Assay	Concentration in 20 µL Sample	Concentration in 10 µL Sample	Result
Sodium chloride	10 mM	100 mM	200 mM	OK
Magnesium chloride	2 mM	20 mM	40 mM	OK †
Sodium acetate	10 mM	100 mM	200 mM	OK †
Ammonium acetate	10 mM	100 mM	200 mM	OK
Potassium phosphate, pH 7.4	5 mM	50 mM	100 mM	OK
Ethanol	1%	10%	20%	OK
Phenol	0.1%	1%	2%	OK †
Chloroform ‡	0.2%	2%	4%	OK
SDS	0.01%	0.1%	0.2%	NR
Triton X-100	0.001%	0.01%	0.02%	OK
dNTPs §	100 µM	1 mM	2 mM	OK
BSA	20 µg/mL	200 µg/mL	400 µg/mL	OK
IgG	10 µg/mL	100 µg/mL	200 µg/mL	OK
ssDNA	1X	1X	1X	OK
Oligos	1X	1X	1X	OK
dsDNA	1X	1X	1X	OK

* *E.coli* rRNA standards were assayed in the presence or absence of contaminants at the indicated final concentrations. Equivalent concentrations (approximate) in 20 µL or 10 µL sample volumes are also listed. Results are given either as OK, usually less than 10% perturbation, or as NR, not recommended. † An acceptable result, but with some distortion of the standard curve; for best results, add the same amount of contaminant to the standard samples. ‡ Immiscible. § A mixture of dATP, dCTP, dGTP, and dTTP.

Qubit™ Assay Kits Compatible with the Qubit® 2.0 Fluorometer

A number of fluorescence-based quantitation kits are available for use with the Qubit® 2.0 Fluorometer. Table 3 will help you choose a kit based on the target molecule being measured and the number of assays you require.

Table 3. Qubit™ Assay Kits for use with the Qubit® 2.0 Fluorometer.

Product	Cat. no.	Number of Assays*	Target	Notes
Qubit™ dsDNA BR Assay Kit	Q32850	100	dsDNA	• core range (high confidence): 0.01 µg/mL to 5 µg/mL† • extended range (moderate confidence): 5 µg/mL to 10 µg/mL† • useful for quantitation of genomic and miniprep DNA samples • accurate in the presence of RNA, salts, solvents, proteins, and free nucleotides
Qubit™ dsDNA BR Assay Kit	Q32853	500		
Qubit™ dsDNA HS Assay Kit	Q32851	100	dsDNA	• core range (high confidence): 1 ng/mL to 500 ng/mL† • extended ranges (moderate confidence): 0.5 ng/mL to 1 ng/mL and 500 ng/mL to 600 ng/mL† • useful for quantitation of PCR products, viral DNA, and samples for subcloning • accurate in the presence of RNA, salts, solvents, proteins, and free nucleotides
Qubit™ dsDNA HS Assay Kit	Q32854	500		
Qubit™ ssDNA Assay Kit	Q10212	100	ssDNA	• core range (high confidence): 5 ng/mL to 1,000 ng/mL† • extended ranges (moderate confidence): 1 ng/mL to 5 ng/mL and 1,000 ng/mL to 1,200 ng/mL† • useful for quantitation of oligos, primers, denatured DNA, PCR products • accurate in the presence of salts, urea, solvents, proteins, ATP, and agarose
Qubit™ RNA Assay Kit	Q32852	100	RNA	• core range (high confidence): 25 ng/mL to 500 ng/mL† • extended ranges (moderate confidence): 20 ng/mL to 25 ng/mL and 500 ng/mL to 1,000 ng/mL† • useful for quantitation of samples for microarray, RT-PCR, and Northern blot procedures • accurate in the presence of DNA, salts, solvents, proteins, and free nucleotides
Qubit™ RNA Assay Kit	Q32855	500		
Qubit™ RNA BR Assay Kit	Q10210	100	RNA	• core range (high confidence): 0.1 µg/mL to 5 µg/mL† • extended ranges (moderate confidence): 0.05 µg/mL to 0.1 µg/mL and 5–6 µg/mL† • useful for quantitation of samples for microarray, RT-PCR, and Northern blot procedures • accurate in the presence of DNA, salts, solvents, proteins, and free nucleotides
Qubit™ RNA BR Assay Kit	Q10211	500		
Qubit™ Protein Assay Kit	Q33211	100	protein	• core range (high confidence): 1.25 µg/mL to 25 µg/mL† • extended ranges (moderate confidence): 1 µg/mL to 1.25 µg/mL and 25 µg/mL to 26 µg/mL† • little protein-to-protein difference in signal • accurate in the presence of DTT, β-mercaptoethanol, amino acids, and DNA • signal is stable for 3 hours
Qubit™ Protein Assay Kit	Q33212	500		

*Based on an assay volume of 200 µL. †Concentration ranges refer to the concentration of sample after dilution in the assay tube.

Product List **Current prices may be obtained from our website or from our Customer Service Department.**

Cat. no.	Product Name	Unit Size
Q32852	Qubit™ RNA Assay Kit, 100 assays *5–100 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q32855	Qubit™ RNA Assay Kit, 500 assays *5–100 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Related products		
Q10210	Qubit™ RNA BR Assay Kit, 100 assays *20–1000 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q10211	Qubit™ RNA BR Assay Kit, 500 assays *20–1000 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q10212	Qubit™ ssDNA Assay Kit, 100 assays *1–200 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q32850	Qubit™ dsDNA BR Assay Kit, 100 assays *2–1000 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q32853	Qubit™ dsDNA BR Assay Kit 500 assays *2–1000 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q32851	Qubit™ dsDNA HS Assay Kit, 100 assays *0.2–100 ng* *for use with the Qubit® 2.0 Fluorometer	1 kit
Q32854	Qubit™ dsDNA HS Assay Kit, 500 assays *0.2–100 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q33211	Qubit™ Protein Assay Kit, 100 assays *0.25–5 µg* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q33212	Qubit™ Protein Assay Kit, 500 assays *0.25–5 µg* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q32856	Qubit™ assay tubes *set of 500*	1 set
Q32866	Qubit® 2.0 Fluorometer	each
Q32867	Qubit® 2.0 Fluorometer USB	each
Q32868	Qubit® 2.0 Fluorometer International Power Cord (replacement)	each

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Qubit™ RNA BR Assay Kits

For use with the Qubit® 2.0 Fluorometer

Table 1. Contents and storage information.

Material	Amount		Concentration	Storage	Stability
	Q10210 (100 assays)	Q10211 (500 assays)			
Qubit™ RNA BR Reagent (Component A)	250 µL	1.25 mL	200X concentrate in DMSO	• ≤ 25°C • Desiccate • Protect from light	When stored as directed, kits are stable for 6 months.
Qubit™ RNA BR Buffer (Component B)	50 mL	250 mL	Not applicable	≤ 25°C	
Qubit™ RNA BR Standard #1 (Component C)	1 mL	5 mL	0 ng/µL in TE buffer	• 2–6°C • Do not freeze	
Qubit™ RNA BR Standard #2 (Component D)	4 × 250 µL	10 × 500 µL	100 ng/µL in TE buffer		

Introduction

The Qubit™ RNA BR (Broad-Range) Assay Kits for use with the Qubit® 2.0 Fluorometer make RNA quantitation easy and accurate. The kit provides concentrated assay reagent, dilution buffer, and prediluted RNA standards. Simply dilute the reagent using the buffer provided, add your sample (any volume between 1 µL and 20 µL is acceptable), and read the concentration using the Qubit® 2.0 Fluorometer. The assay is highly selective for RNA over double-stranded DNA (dsDNA) (*Appendix*, Figure 1) and is accurate for initial sample concentrations from 1 ng/µL to 1 µg/µL providing an assay range from 20–1,000 ng. The assay is performed at room temperature, and the signal is stable for 3 hours. Common contaminants, such as salts, free nucleotides, solvents, detergents, or protein are well tolerated in the assay (*Appendix*, Table 2). In addition to the Qubit™ RNA BR Assay Kits described here, we also offer other kits for assaying DNA and protein (*Appendix*, Table 3).

To determine the purity of your sample, use the Qubit™ RNA BR Assay Kit together with the Qubit™ dsDNA BR Assay Kit. These measurements give you a much better indication of sample purity than that produced by measuring the A_{260}/A_{280} ratio. To measure protein contamination in nucleic acid samples, simply run 1–20 µL of the sample in the Qubit™ protein assay.

Note: All Qubit™ assay kits can also be used with the Qubit® 1.0 Fluorometer.

Before You Begin

Materials Required but Not Provided

- Plastic container (disposable) for mixing the Qubit™ working solution (step 1.3)
- Qubit® assay tubes (500 tubes, Cat. no. Q32856) or Axygen PCR-05-C tubes (VWR, part no. 10011-830)

Storing the Qubit™ RNA Assay Kits

The Qubit™ RNA reagent and buffer are designed for room temperature storage. The Qubit™ RNA reagent is supplied in DMSO, which freezes at temperatures lower than room temperature. Store the RNA standards at 4°C.

The Qubit™ RNA reagent is sensitive to light. Store the vial **in the dark** when not in use.

Critical Assay Parameters

Assay Temperature

The Qubit™ RNA assay for the Qubit® 2.0 Fluorometer delivers optimal performance when all solutions are at room temperature (22–28˚C). The Qubit™ assays are designed to be performed at room temperature, and temperature fluctuations can influence the accuracy of the assay (*Appendix*, Figure 2). To minimize temperature fluctuations, store the Qubit™ RNA reagent and the Qubit™ RNA buffer at room temperature and insert all assay tubes into the Qubit® 2.0 Fluorometer only for as much time as it takes for the instrument to measure the fluorescence, because the Qubit® 2.0 Fluorometer can raise the temperature of the assay solution significantly, even over a period of a few minutes. Do not hold the assay tubes in your hand before reading, as this will warm the solution and result in a low reading.

Incubation Time

To allow the Qubit™ assay to reach optimal fluorescence, incubate the tubes for the DNA and RNA assays for 2 minutes after mixing the sample or standard with the working solution. After this incubation period, the fluorescence signal is stable for 3 hours at room temperature.

Photobleaching of the Qubit™ Reagent

The Qubit™ reagents exhibit high photostability in the Qubit® 2.0 Fluorometer, showing <0.3% drop in fluorescence after 9 readings and <2.5% drop in fluorescence after 40 readings. It is important to remember, however, that if the assay tube remains in the Qubit® 2.0 Fluorometer for multiple readings, a temporary reduction in fluorescence will be observed as the solution increases in temperature (see *Appendix*, Figure 2). Note that the temperature inside the Qubit® 2.0 Fluorometer may be as much as 3°C above room temperature after 1 hour. For this reason, if you want to perform multiple readings of a single tube, remove the tube from the instrument and let it equilibrate to room temperature for 30 seconds before taking another reading.

Calibrating the Qubit® 2.0 Fluorometer

For each assay, you have the choice to run a new calibration or to use the values from the previous calibration. As you first use the instrument, perform a new calibration each time. As you become familiar with the assays, the instrument, your pipetting accuracy, and significant temperature fluctuations within your laboratory, determine the level of comfort you have using the calibration data stored in from the last time the instrument was calibrated. Remember also that the fluorescence signal in the tubes containing standards and the samples is stable for not longer than 3 hours. See Figure 3 in the *Appendix* for an example of the calibration curve used to generate the quantitation results.

RNAse-free Handling

The calibration standards included in the Qubit™ RNA Assay Kit are high-quality rRNA standards. The integrity and concentration of these standards is critical to the optimal performance of the Qubit™ RNA BR assay. As such, we highly recommend treating the rRNA standards as you would any other precious RNA. Use appropriate RNAse-free handling techniques, including RNAse-free gloves, pipette tips, and tubes. Keep the tube lids closed whenever possible; do not touch the pipet to the inside wall of the tube when withdrawing a sample, and return the rRNA standard to the refrigerator as soon as possible after use.

Handling and Disposal

No data are currently available addressing the mutagenicity or toxicity of the Qubit™ RNA BR reagent (Component A). This reagent is known to bind nucleic acid and is provided as a solution in DMSO. Treat the Qubit™ RNA BR reagent with the same safety precautions as all other potential mutagens and dispose of the dye in accordance with local regulations.

Experimental Protocol

Performing the Qubit™ RNA BR Assay

The protocol below assumes you are preparing standards for calibrating the Qubit® 2.0 Fluorometer. If you plan to use the last calibration performed on the instrument, you need fewer tubes (step 1.1) and less working solution (step 1.3). More detailed instructions on the use of the Qubit® 2.0 Fluorometer (corresponding to steps 1.9–1.15 and 2.1–2.6) can be found in the user manual accompanying the instrument. For sample purity determinations, it is possible to use the Qubit® 2.0 Fluorometer to calculate the amount of dsDNA and RNA in the same sample. Simply perform each assay for your sample.

1.1 Set up the required number of 0.5 mL tubes you need for standards and samples. The Qubit™ RNA BR assay requires 2 standards.

Note: Use only thin-wall, clear 0.5 mL optical-grade real-time PCR tubes. Acceptable tubes include Qubit® assay tubes (500 tubes, Invitrogen Cat. no. Q32856) or Axygen PCR-05-C tubes (VWR, part number 10011-830).

1.2 Label the tube lids.

1.3 Make the Qubit™ working solution by diluting the Qubit™ RNA BR reagent 1:200 in Qubit™ RNA BR buffer. Use a clean plastic tube each time you make Qubit™ working solution. **Do not mix the working solution in a glass container.**

Note: The final volume in each tube must be 200 µL. Each standard tube requires 190 µL of Qubit™ working solution, and each sample tube requires anywhere from 180 µL to 199 µL. Prepare sufficient Qubit™ working solution to accommodate all standards and samples.

For example, for 8 samples, prepare enough working solution for the samples and 2 standards: ~200 µL per tube in 10 tubes yields 2 mL of working solution (10 µL of Qubit™ reagent plus 1,990 µL of Qubit™ buffer).

1.4 Load 190 µL of Qubit™ working solution into each of the tubes used for standards.

1.5 Add 10 µL of each Qubit™ RNA BR standard to the appropriate tube and mix by vortexing 2–3 seconds, being careful not to create bubbles.

Note: Careful pipetting is critical to ensure that exactly 10 µL of each Qubit™ RNA BR standard is added to 190 µL of Qubit™ working solution. It is also important to label the lid of each standard tube correctly as calibration of the Qubit® 2.0 Fluorometer requires that the standards be introduced to the instrument in the right order.

1.6 Load Qubit™ working solution into individual assay tubes so that the final volume in each tube after adding sample is 200 µL.

Note: Your sample can be anywhere between 1 µL and 20 µL, therefore, load each assay tube with a volume of Qubit™ working solution anywhere between 180 µL and 199 µL.

1.7 Add each of your samples to assay tubes containing the correct volume of Qubit™ working solution (prepared in step 1.6) and mix by vortexing 2–3 seconds. The final volume in each tube should be 200 µL.

1.8 Allow all tubes to incubate at room temperature for 2 minutes.

1.9 On the Home Screen of the Qubit® 2.0 Fluorometer, press **RNA**, and then select **RNA Broad Range** as the assay type. The Standards Screen is automatically displayed.

Note: If you have already performed a calibration for the selected assay, Qubit® 2.0 Fluorometer will prompt you to choose between reading new standards and using the previous calibration. See *Calibrating the Qubit® 2.0 Fluorometer* above for calibration guidelines.

1.10 On the Standards Screen, press **Yes** to run a new calibration or press **No** to use the last calibration.

1.11 If you pressed **No** on the Standards Screen, proceed to step 1.12. If you selected **Yes** to a run new calibration, follow instructions below.

Running a New Calibration

Insert the tube containing Standard #1 in the Qubit® 2.0 Fluorometer, close the lid, and press **Read**. The reading will take approximately 3 seconds.
Remove Standard #1.
Insert the tube containing Standard #2 in the Qubit® 2.0 Fluorometer, close the lid, and press **Read**.
Remove Standard #2.

1.12 If you pressed **No** on the Standards Screen, the Sample Screen will be automatically displayed. Insert a sample tube into the Qubit® 2.0 Fluorometer, close the lid, and press **Read**.

1.13 Upon the completion of the measurement, the result will be displayed on the screen.

Note: The value given by the Qubit® 2.0 Fluorometer at this stage corresponds to the concentration after your sample was diluted into the assay tube. You can record this value and perform the calculation yourself to find out the concentration of your original sample (see *Calculating the Concentration of Your Sample*, next page) or the Qubit® 2.0 Fluorometer performs this calculation for you (see *Dilution Calculator*, next page).

1.14 To read the next sample, remove the sample from the Qubit® 2.0 Fluorometer, insert the next sample, and press **Read Next Sample**.

1.15 Repeat sample readings until all samples have been read.

Calculating the Concentration of Your Sample

The Qubit® 2.0 Fluorometer gives values for the Qubit™ RNA BR assay in µg/mL. This value corresponds to the concentration after your sample was diluted into the assay tube. To calculate the concentration of your sample, use the following equation:

$$\text{Concentration of your sample} = \text{QF value} \times \left(\frac{200}{x}\right)$$

where QF value = the value given by the Qubit® 2.0 Fluorometer
x = the number of microliters of sample you added to the assay tube

This equation generates a result with the same units as the value given by the Qubit® 2.0 Fluorometer (*i.e.*, if the Qubit® 2.0 Fluorometer gave a concentration in µg/mL, the result of the equation will be in µg/mL).

Dilution Calculator

The "Dilution Calculator" feature of the Qubit® 2.0 Fluorometer calculates the concentration of your original sample based on the volume of sample you have added to the assay tube. To have the Qubit® 2.0 Fluorometer perform this calculation for you, follow the instruction below.

2.1 Upon completion of the sample measurement, press **Calculate Stock Conc.** The Dilution Calculator Screen containing the volume roller wheel is displayed.

2.2 Using the volume roller wheel, select the volume of your original sample that you have added to the assay tube. When you stop scrolling, the Qubit® 2.0 Fluorometer calculates the original sample concentration based on the measured assay concentration.

2.3 To change the units in which the original sample concentration is displayed, press **ng/mL**. A pop-up window showing the current unit selection (as indicated by an adjacent red dash) opens.

2.4 Select the unit for your original sample concentration by touching the desired unit in the unit selection pop-up window. To close the unit selection pop-up window, touch anywhere on the screen outside the pop-up.

The Qubit® 2.0 Fluorometer automatically converts the units to your selection once the unit selection pop-up window is closed.

Note: The unit button next to your sample concentration reflects the change in the units (e.g., if you change the unit to pg/µL, the button will display pg/µL).

2.5 To save the data from your calculation to the Qubit® 2.0 Fluorometer, press **Save** on the Dilution Calculator screen. The last calculated value of your measurement will be saved in the .CSV file and tagged with a time and date stamp.

2.6 To exit the Dilution Calculator Screen, press any navigator button on the bottom of the screen or Read Next Sample.

Note: When you navigate away from the Dilution Calculator screen, the Qubit® 2.0 Fluorometer saves the last values for the sample volume and the units in the Dilution Calculator screen only. Returning to the Dilution Calculator screen displays these last selected values.

Appendix

Selectivity of the Qubit™ RNA BR Assay

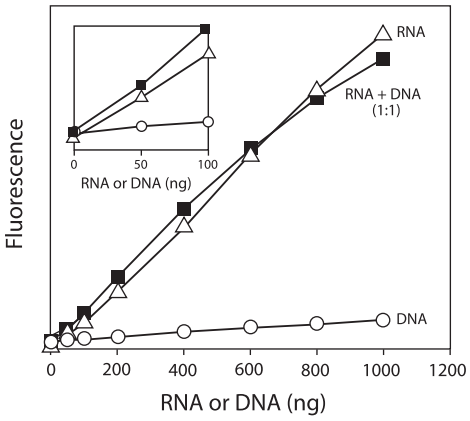

Figure 1. RNA selectivity and sensitivity of the Qubit™ RNA BR assay (Q10210, Q10211). Triplicate 10 µL samples of *E. coli* rRNA (△), λ DNA (○), or a 1:1 mixture of RNA and DNA (■) were assayed in the Qubit™ RNA BR assay. Fluorescence was measured at 630/660 nm and plotted versus the mass of nucleic acid for the RNA alone or DNA alone, or versus the mass of the RNA component in the 1:1 mixture. The variation (CV) of replicate RNA determinations was ≤10%. The inset is an enlargement of the graph to show the sensitivity of the assay for RNA. Background fluorescence has not been subtracted.

Effect of Temperature on the Qubit™ RNA Assay

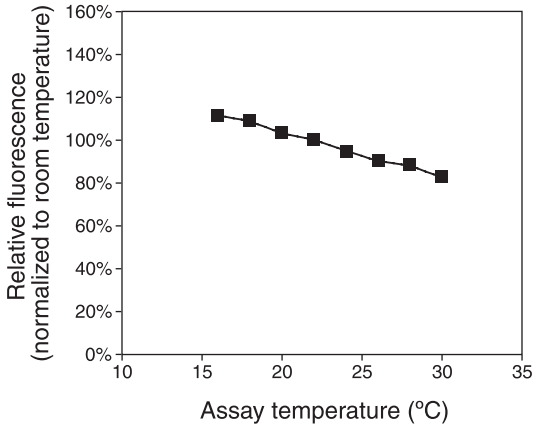

Figure 2. Plot of fluorescence vs. temperature for the Qubit™ RNA assay. The Qubit™ assays were designed to be performed at room temperature, as temperature fluctuations can influence the accuracy of the assay.

How the Qubit® 2.0 Fluorometer Calculates Concentration

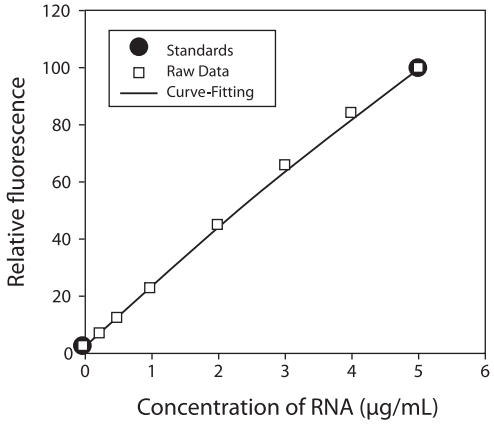

Figure 3. The curve-fitting algorithm used to determine concentration in the Qubit™ RNA BR assay. The Qubit® 2.0 Fluorometer generates concentration data based on the relationship between the two standards used in calibration. This plot shows the line corresponding to the curve-fitting algorithm (a modified Hill plot) used in the calculation of concentration data for the Qubit™ RNA BR assay. For reference, the positions of the standards and a set of data points from an actual experiment are shown superimposed onto the line, demonstrating that the curve-fitting algorithm gives accurate values for quantitation.

Contaminants Tolerated by the Qubit™ RNA BR Assay

Table 2. Effect of contaminants in the Qubit™ RNA BR assay, tested over the range 500–5,000 ng/mL.*

Contaminant	Final Concentration in the Assay	Concentration in 20 µL Sample	Concentration in 10 µL Sample	Result
Sodium chloride	10 mM	100 mM	200 mM	OK
Magnesium chloride	2 mM	20 mM	40 mM	OK†
Sodium acetate	10 mM	100 mM	200 mM	OK†
Ammonium acetate	10 mM	100 mM	200 mM	OK
Potassium phosphate, pH 7.4	5 mM	50 mM	100 mM	OK
Ethanol	0.1%	1%	2%	OK
Phenol	0.1%	1%	2%	OK†
Chloroform‡	0.2%	2%	4%	OK
SDS	0.01%	0.1%	0.2%	NR
Triton® X-100	0.001%	0.01%	0.02%	OK
dNTPs§	100 µM	1 mM	2 mM	OK
BSA	20 µg/mL	200 µg/mL	400 µg/mL	OK
IgG	10 µg/mL	100 µg/mL	200 µg/mL	OK
ssDNA	1X	1X	1X	OK
Oligos	1X	1X	1X	OK
dsDNA	1X	1X	1X	OK

**E.coli* rRNA standards were assayed in the presence or absence of contaminants at the indicated final concentrations. Equivalent concentrations (approximate) in 20 µL or 10 µL sample volumes are also listed. Results are given either as OK, usually less than 10% perturbation, or as NR, not recommended. †An acceptable result, but with some distortion of the standard curve; for best results, add the same amount of contaminant to the standard samples. ‡Immiscible. §A mixture of dATP, dCTP, dGTP, and dTTP.

Qubit™ Assay Kits Compatible with the Qubit® 2.0 Fluorometer

A number of fluorescence-based quantitation kits are available for use with the Qubit® 2.0 Fluorometer. Table 3 helps you choose a kit based on the target molecule being measured and the number of assays you require.

Table 3. Qubit™ Assay Kits for use with the Qubit® 2.0 Fluorometer.

Product	Cat. no.	Number of Assays*	Target	Notes
Qubit™ dsDNA BR Assay Kit	Q32850	100	dsDNA	• core range (high confidence): 0.01 µg/mL to 5 µg/mL† • extended range (moderate confidence): 5 µg/mL to 10 µg/mL† • useful for quantitation of genomic and miniprep DNA samples • accurate in the presence of RNA, salts, solvents, proteins, and free nucleotides
Qubit™ dsDNA BR Assay Kit	Q32853	500		
Qubit™ dsDNA HS Assay Kit	Q32851	100	dsDNA	• core range (high confidence): 1 ng/mL to 500 ng/mL† • extended ranges (moderate confidence): 0.5 ng/mL to 1 ng/mL and 500 ng/mL to 600 ng/mL† • useful for quantitation of PCR products, viral DNA, and samples for subcloning • accurate in the presence of RNA, salts, solvents, proteins, and free nucleotides
Qubit™ dsDNA HS Assay Kit	Q32854	500		
Qubit™ ssDNA Assay Kit	Q10212	100	ssDNA	• core range (high confidence): 5 ng/mL to 1,000 ng/mL† • extended ranges (moderate confidence): 1 ng/mL to 5 ng/mL and 1,000 ng/mL to 1,200 ng/mL† • useful for quantitation of oligos, primers, denatured DNA, PCR products • accurate in the presence of salts, urea, solvents, proteins, ATP, and agarose
Qubit™ RNA Assay Kit	Q32852	100	RNA	• core range (high confidence): 25 ng/mL to 500 ng/mL† • extended ranges (moderate confidence): 20 ng/mL to 25 ng/mL and 500 ng/mL to 1,000 ng/mL† • useful for quantitation of samples for microarray, RT-PCR, and Northern blot procedures • accurate in the presence of DNA, salts, solvents, proteins, and free nucleotides
Qubit™ RNA Assay Kit	Q32855	500		
Qubit™ RNA BR Assay Kit	Q10210	100	RNA	• core range (high confidence): 0.1 µg/mL to 5 µg/mL† • extended ranges (moderate confidence): 0.05 µg/mL to 0.1 µg/mL and 5–6 µg/mL† • useful for quantitation of samples for microarray, RT-PCR, and Northern blot procedures • accurate in the presence of DNA, salts, solvents, proteins, and free nucleotides
Qubit™ RNA BR Assay Kit	Q10211	500		
Qubit™ Protein Assay Kit	Q33211	100	protein	• core range (high confidence): 1.25 µg/mL to 25 µg/mL† • extended ranges (moderate confidence): 1 µg/mL to 1.25 µg/mL and 25 µg/mL to 26 µg/mL† • little protein-to-protein difference in signal • accurate in the presence of DTT, β-mercaptoethanol, amino acids, and DNA • signal is stable for 3 hours
Qubit™ Protein Assay Kit	Q33212	500		

*Based on an assay volume of 200 µL. †Concentration ranges refer to the concentration of sample after dilution in the assay tube.

Product List **Current prices may be obtained from our website or from our Customer Service Department.**

Cat. no.	Product Name	Unit Size
Q10210	Qubit™ RNA BR Assay Kit, 100 assays *20–1000 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q10211	Qubit™ RNA BR Assay Kit, 500 assays *20–1000 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Related products		
Q32852	Qubit™ RNA Assay Kit, 100 assays *5–100 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q32855	Qubit™ RNA Assay Kit, 500 assays *5–100 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q10212	Qubit™ ssDNA Assay Kit, 100 assays *1–200 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q32850	Qubit™ dsDNA BR Assay Kit, 100 assays *2–1000 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q32853	Qubit™ dsDNA BR Assay Kit 500 assays *2–1000 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q32851	Qubit™ dsDNA HS Assay Kit, 100 assays *0.2–100 ng* *for use with the Qubit® 2.0 Fluorometer	1 kit
Q32854	Qubit™ dsDNA HS Assay Kit, 500 assays *0.2–100 ng* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q33211	Qubit™ Protein Assay Kit, 100 assays *0.25–5 µg* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q33212	Qubit™ Protein Assay Kit, 500 assays *0.25–5 µg* *for use with the Qubit® 2.0 Fluorometer*	1 kit
Q32856	Qubit™ assay tubes *set of 500*	1 set
Q32866	Qubit® 2.0 Fluorometer	each
Q32867	Qubit® 2.0 Fluorometer USB	each
Q32868	Qubit® 2.0 Fluorometer International Power Cord (replacement)	each

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